Haematopoietic and immune defects associated with GATA2 mutation

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Summary
Heterozygous familial or sporadic GATA2 mutations cause a multifaceted disorder, encompassing susceptibility to infection, pulmonary dysfunction, autoimmunity, lymphoedema and malignancy. Although often healthy in childhood, carriers of defective GATA2 alleles develop progressive loss of mononuclear cells (dendritic cells, monocytes, B and Natural Killer lymphocytes), elevated FLT3 ligand, and a 90% risk of clinical complications, including progression to myelodysplastic syndrome (MDS) by 60 years of age. Premature death may occur from childhood due to infection, pulmonary dysfunction, solid malignancy and MDS/acute myeloid leukaemia. GATA2 mutations include frameshifts, amino acid substitutions, insertions and deletions scattered throughout the gene but concentrated in the region encoding the two zinc finger domains. Mutations appear to cause haplo-insufficiency, which is known to impair haematopoietic stem cell survival in animal models. Management includes genetic counselling, prevention of infection, cancer surveillance, haematopoietic monitoring and, ultimately, stem cell transplantation upon the development of MDS or another life-threatening complication.

Keywords: GATA2, bone marrow failure, immunodeficiency.

GATA binding protein 2 (GATA2) is a key transcriptional regulator of haematopoiesis required for the development and maintenance of a healthy stem cell pool. Mutation of GATA2 has long been predicted to be relevant to leukaemogenesis but the human syndromes of GATA2 deficiency have only been recently described. Clinical phenotypes include patients with hereditary myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) and also protean manifestations of immunodeficiency, neoplasia, lymphoedema and extra-haematopoietic defects.

In this review we summarize the molecular biology, clinical, haematological and immunological features that arise and discuss potential strategies for clinical management.

GATA2 gene structure and regulation
GATA2 is one of six GATA binding-factors that regulate gene expression by binding to the DNA motif GATA and other transcription factors via two zinc finger domains (Orkin, 2000; Bresnick et al, 2010; Rodrigues et al, 2012). In the embryo, GATA2 is pivotal in the endothelial to haematopoietic transition that produces the first adult haematopoietic stem cells (HSCs) and consequently, homozygous knock-out is lethal due to the failure of definitive haematopoiesis (Tsai et al, 1994). In adult haematopoiesis, GATA2 is required for HSC survival and self-renewal, interacting with a complex network of transcription factors that specify early lineage commitment, including SPI1 (PU.1), FLI1, TAL1 (SCL), LMO2 and RUNX1, among others (Dore et al, 2012; Beck et al, 2013; May et al, 2013). During haematopoietic differentiation, GATA2 is likely to play a key role in downstream fate decisions together with CEBPs, GATA1 and SPI1 and is expressed in mature megakaryocytes, mast cells and monocytes (Fig 1).

The GATA2 gene is situated on the long arm of human chromosome 3 at position 21.3 and its expression is regulated at multiple levels. Enhancers at −110 kb (77 kb in mouse) and in intron 5 (intron 4 in mouse) are required for appropriate haematopoietic expression (Martowicz et al, 2005; Grass et al, 2006; Khandekar et al, 2007; Brandt et al, 2008). Multiple binding sites for GATA1 and GATA2 are found within the regulatory regions of GATA2, including the −110 kb and +9.5 kb intronic enhancers (Martowicz et al, 2005; Grass et al, 2006; Khandekar et al, 2007; Snow et al, 2011; Lim et al, 2012; Gao et al, 2013). The intronic enhancer contains an E box GATA composite element, which mediates assembly of a complex containing GATA1 or GATA2, TAL1, LIM domain binding protein (LDB1) and LIM domain only 2 (LMO2). SMARCA4 (BRG1), the ATPase component of the SWI/SNF complex, may also act with LDB1 to establish and maintain GATA2 expression by keeping the +9.5 site in open chromatin configuration (Sanalkumar et al, 2014). In addition, GATA2 transcription
Stem cell performance of HSCs in serial or competitive transplantation in animal models. The production of mouse HSCs and allele, or haplo-insufficiency, induces defects of haematopoiesis. GATA factors can bind to DNA as monomers or dimers and their configuration is likely to be concentration-dependent (Bates et al., 2008). Inactivation of one GATA2 allele, or haplo-insufficiency, induces defects of haematopoiesis in animal models. The production of mouse HSCs and performance of HSCs in serial or competitive transplantation assays is inferior and there is perturbation of the granulocyte-macrophage colony-forming unit compartment (Ling et al., 2004; Rodrigues et al., 2005, 2008). Insufficient GATA2 appears to allow HSCs to enter cell cycle and differentiate, thereby depleting self-renewal capacity (Exso et al., 2002; de Pater et al., 2013), while over-expression impairs haematopoiesis by blocking differentiation (Heyworth et al., 1999; Persons et al., 1999; Tipping et al., 2009). Fine-tuning of the balance between self-renewal and differentiation of HSC appears to be a critical function of GATA2, possibly through its role in mediating contact-dependent quiescence signals from the BM niche (de Pooter et al., 2006; Guiu et al., 2013). Direct effects on apoptosis are also thought to be mediated by an interaction of GATA2 with BCL2L1 (BCL-XL) (Rodrigues et al., 2005). The consequences of GATA2 haplo-insufficiency upon HSC equilibrium are more strikingly revealed in humans than mice, owing to the greater longevity of haematopoiesis.

**Haplo-insufficiency of GATA2**

The level of expression of GATA2 relative to other transcription factors is important in gene regulation and cell fate decisions. GATA factors can bind to DNA as monomers or dimers and their configuration is likely to be concentration-dependent (Bates et al., 2008). Inactivation of one GATA2 allele, or haplo-insufficiency, induces defects of haematopoiesis in animal models. The production of mouse HSCs and performance of HSCs in serial or competitive transplantation

![Diagram of GATA2 interactions](image)

**Fig 1. Role of GATA2 in haematopoietic differentiation.** Simplified map of key interactions of GATA2 with selected major lineage-specifying transcription factors. The factors indicated are necessary for downstream differentiation according to knock-out factors or are highly expressed in differentiated cells but the figure does not contain an exhaustive list of all the transcription factors that have been implicated. Antagonism between pairs of transcription factors is a notable feature of fate decisions, as exemplified by GATA2 and SPI1 (PU.1) in influencing the spectrum of early commitment. In the ‘GATA switch’ GATA2 is replaced by GATA1 at erythroid-specific sites. For more details and source information see: (Orkin, 2000; Orkin & Zon, 2008; Bresnick et al., 2010; Rodrigues et al., 2012).

is regulated by several loci including CEBPA, HOXA9, ETS1, BMP4, NOTCH1, SPI1 and EVI1 and by cytokines IL1 and TNFα (Vicente et al., 2012).

GATA2 cooperates with six other factors (TAL1, LYL1, LMO2, ERG, FLI1 and RUNX1) to form a core heptad regulatory unit bound to over 1000 loci in primitive haematopoietic cells (Wilson et al., 2010). Heptad target genes include microRNAs and are lineage and maturation stage-specific. Direct targets of GATA2 itself include, GATA1, SPI1 and CEBPA, together with the heptad factors TAL1, LMO2, FLI1 and RUNX1. The GATA2 protein interacts directly with ZFPM1 (FOG1), SPI1 and CEBPA (Vicente et al., 2012).

Three GATA2 transcripts have been described. Expression of the distal first exon, IS, is haematopoietic-restricted and involved in specification of definitive HSCs during embryogenesis (Minegishi et al., 1999; Pan et al., 2000; Kobayashi-Osaki et al., 2005). Two protein isoforms have been described; isoform 1 with 480 residues and a shorter isoform 2 which is truncated by 14 residues at the second zinc finger, due to alternative splicing of the last exon. GATA2 may be modified by phosphorylation, acetylation and sumoylation and is rapidly turned over by ubiquitination (Towatari et al., 1995; Hayakawa et al., 2004; Minegishi et al., 2005).

**Heterozygous mutation of GATA2 in humans**

Nearly 100 GATA2 mutations have been described, either as germ-line genetic defects or somatic mutations in association with other drivers, such as biallelic CEBPA mutation in AML (Fig 2, Tables I and SI). Approximately one-third of all germ-line mutations are inherited and the rest occur de novo. These include a small number of whole gene deletions and 29 frame-shift or nonsense mutations, distributed from the initiation site to the end of the second zinc finger. A further 11 in-frame insertions or deletions and 54 single nucleotide variants causing amino acid substitution are concentrated in exons 3, 4 and 5, encoding the two zinc finger domains. Splice site mutations are also found between coding exons 3 and 4. Two discrete mutations of the intron 5 enhancer, predicted to affect transcription factor binding, have also been reported. Overall, approximately two-thirds of all cases
described have mutations in the zinc finger domains (Fig 2). No mutations have been observed in the 5' or 3' untranslated regions (UTRs) or in the distal section of the last exon, beyond the region encoding the second zinc finger. In order to capture all of the reported single base changes, small insertions and deletions, it is necessary to sequence codons 1–398, together with the intron 5 enhancer.

Although more than half the variants described are single amino acid substitutions that may lead to the translation of mutated protein with altered function, there is reasonable
expectation that the functional effects of heterozygous mutation are primarily due to haplo-insufficiency (Table I). The main argument is that gene deletions and frame-shift mutations that are null alleles, lead to virtually the same constellation of phenotypes as amino acid substitution variants. Many single amino acid substitutions are predicted to significantly impair DNA binding of the zinc fingers potentially making them functionally inactive (Dickinson et al, 2011). However, it is also possible that these mutants have residual function or can even act in a dominant negative fashion, as reported for T354M (Hahn et al, 2011). Gain of function is reported for the L359V variant found in blast transformation of chronic myeloid leukaemia (CML) (Zhang et al, 2008).

Of all the known regulatory regions of GATA2, only the intron 5 enhancer has been reported to contain germ-line mutations (Johnson et al, 2012; Hsu et al, 2013). This enhancer has been shown to be critical for GATA2 expression in endothelium and HSC (Khandekar et al, 2007; Johnson et al, 2012; Lim et al, 2012; Gao et al, 2013). The existence of several patients with reduced GATA2 due to allelic expression imbalance suggests that other regulatory sites will be involved but sequencing the 5' enhancers of GATA2 has so far proven unfruitful (Hsu et al, 2013; unpublished observations). A recent report in sporadic AML invokes hypermethylation as a potential mechanism of loss of GATA2 expression (Celton et al, 2014). Regulation of GATA2 translation by MIR23A binding to the 3'-UTR has also recently been described as the mechanism by which SON protein enhances GATA2 expression (Ahn et al, 2013).

Clinical syndromes associated with GATA2 mutation

The clinical syndromes of human GATA2 deficiency were uncovered by four independent groups, each working with a different focus. Monocytopenia with susceptibility to atypical mycobacterial infection, such as mycobacterium avium complex, was described as 'monoMAC' (Vinh et al, 2010) and GATA2 mutation was revealed by a candidate sequencing approach (Hsu et al, 2011). Loss of dendritic cells (DCs), monocytes B and Natural Killer (NK) lymphoid cells (DCML deficiency) was described in four individuals (Bigley et al, 2011) all found to be harbouring GATA2 mutation by exome sequencing (Dickinson et al, 2011). These studies were closely followed by two groups who had carefully curated cohorts of familial MDS/AML and hereditary lymphoedema with MDS (Emberger syndrome), ultimately localizing the genetic defect to GATA2 (Scott et al, 2010; Hahn et al, 2011; Ostergaard et al, 2011). In all familial cases, the trait was inherited in an autosomal dominant fashion.

A succession of follow-up reports established new cases and recalled a fascinating series of historical precedents by retrospective diagnosis (Kaur et al, 1972; Robinson et al, 1983; Biron et al, 1989; Ballas et al, 1990; Couderc et al, 1992; Horwitz et al, 1996; Wendland et al, 2000; Khanjari et al, 2003; Witzke et al, 2004; Bodor et al, 2012; Holme et al, 2012; Ishida et al, 2012; Kazenwadel et al, 2012; Camargo et al, 2013; Mace et al, 2013; Mutsaers et al, 2013; Niimi et al, 2013; Pasquet et al, 2013; Chou et al, 2014; Gao et al, 2014; West et al, 2014). The first we are aware of was a report in this journal in 1972, describing an Icelandic family with MDS/AML in association with trisomy 8 and Pelger-Huet abnormality (Kaur et al, 1972), subsequently traced two generations later to a GATA2 T354M mutation (Dickinson et al, 2014). Other familial cases of AML with immunodeficiency and pulmonary dysfunction have also since been tracked to GATA2 mutation (Robinson et al, 1983; Horwitz et al, 1996). The original case of human NK deficiency is now known to be a GATA2 phenotype (Biron et al, 1989; Mace et al, 2013). GATA2 mutation has also been identified in paediatric neutropenia and aplastic anaemia (Pasquet et al, 2013).

The protean manifestations of GATA2 mutation and clinical progression of patients have been documented more recently through two larger cohort studies drawn from North America and Europe, summarized in Table II and Fig 3 (Dickinson et al, 2014; Spinner et al, 2014). Penetrance in these selected patients and their family members is more than 90% but with an extended age range of onset from 5 to 55 years and a median survival of 60 years. The most common features are warts from widespread human papilloma virus (HPV) infection, progression to MDS, pulmonary dysfunction including pulmonary alveolar proteinosis (PAP), infection with mycobacteria or fungi and lymphoedema. It is notable that many recognizable features of GATA2 deficiency were described in the early case reports.

Several kindreds have been reported with unaffected individuals carrying GATA2 mutation into their fifth and sixth decades but overall, the lifetime risk of MDS is approximately 90%. By the age of 60 years, the majority of patients will have had additional complications of defective cell-mediated immunity such as warts, herpes virus, mycobacterial or fungal infection. About 20% develop PAP and up to 50% have evidence of pulmonary dysfunction. Malignant disease is common, mostly due to HPV-driven intraepithelial neoplasia but an increase in breast cancer, squamous cell carcinoma and Epstein–Barr virus (EBV) positive neoplasms is reported. Initial reports linking frameshift or null mutations to a higher risk of lymphoedema are supported by larger studies (Hyde & Liu, 2011; Spinner et al, 2014). Severe viral infection is also significantly increased in these individuals. In contrast, MDS and AML appears to be an equal risk with all types of GATA2 mutation (Dickinson et al, 2014; Spinner et al, 2014).

These cohort studies highlight first, that carriers are haematologically normal at birth; second, that the phenotype of mononuclear cytopenia, or DCML deficiency, evolves over time; and third, that loss of mononuclear cells is a common feature of all patients with symptoms (Dickinson et al, 2014; Spinner et al, 2014). In both studies, a simple clinical score
Infection histories and normal levels of class-switched immunoglobulin and memory T cells, as young adults. From a haematological perspective, there are several precedents of hereditary bone marrow failure (BMF) syndromes presenting with an 'accessory phenotype', such as thrombocytopenia in RUNX1 mutation and eosinophilia in CEBPA mutation (Owen et al, 2008; Carmichael et al, 2010). In GATA2 mutation this accessory phenotype appears to be loss of mononuclear cells. It is conceivable that MDS or AML could develop without prior warning from bone marrow (BM) with constitutive GATA2 mutation. However, most hereditary MDS/AML kindreds have younger generations with clear DCML deficiency and MDS and this may have been overlooked in their affected ancestors; parents were reported to have developed AML and deceased grandparents, simply ‘Acute Leukaemia’.

### GATA2 mutation and bone marrow failure

Previously reported associations between susceptibility to mycobacterial infection, PAP and MDS, foreshadow the discovery of GATA2 mutation as a unifying cause. By the age of 60 years, 90% of patients will have developed refractory cytopenia and multilineage dysplasia that meets standard criteria and is often associated with the acquisition of additional genetic defects. Prior to this, the natural history of progressive BMF can be dissected in detail.

As patients with GATA2 mutation progress, a number of features appear that make them readily distinguishable from sporadic MDS (Calvo et al, 2011; Dickinson et al, 2014) (Table III). Cardinal signs are family history of MDS or unusual haematological problem, young age of presentation (median age 21–33 years), atypical infection, severe monocytopenia and a high frequency of hypocellularity, megakaryocyte atypia and fibrosis in the BM. Compared with unselected MDS patients attending outpatients, patients with GATA2 mutation have better preserved haemoglobin, neutrophil and platelet levels but much more severe deficits of mononuclear cells (Calvo et al, 2011; Dickinson et al, 2014; Spinner et al, 2014). Monocytopenia, if present, is highly discriminatory but it can be masked by progenitor or atypical lymphocyte expansion on routine blood counting. In this case, simple lymphocyte subset analysis will reveal striking B and NK deficiency. BM examination may be normal or reveal only slight hypocellularity and megakaryocyte atypia, even when there is gross mononuclear cell deficiency.

An interesting feature of GATA2 mutation is the extremely high elevation of FLT3 ligand. This begins at an early stage and soon separates GATA2 mutation from sporadic MDS. Progressive elevation of FLT3 ligand is associated with worsening cytopenias and clinical complications, suggesting that it may be useful in clinical monitoring (Bigley et al, 2011; Dickinson et al, 2014).

GATA2-deficient BM has been subjected to flow cytometry and clonality studies, revealing profound defects that are not apparent by routine morphological analysis, during early stages of the disease (Bigley et al, 2011; Calvo et al, 2011; Dickinson et al, 2014). In the CD34+ progenitor compartment there is complete absence of the primitive GATA2 mutation and normal levels of class-switched immunoglobulin and memory T cells.
depletion of CD38⁺ granulocytic monocytic progenitors, although sufficient remain to sustain neutrophils in many patients. Clonality, as tested by allele-specific polymerase chain reaction of X chromosome transcripts in females, appears as a relatively early sign, anticipating cellular deficiency. In the early stages of BMF, there is mobilization of CD34⁺ progenitors into the peripheral blood but these appear to fade with increasing hypocellularity and cytopenia before increasing again in the context of leukemic transformation (Bigley et al., 2011; Dickinson et al., 2014). As patients progress, disordered myelopoiesis is evident from the lack of monocyte precursors, hypogranularity of myelocytes and lack of CD16 expression (Calvo et al., 2011).

The gradual and progressive decline in stem cell function in humans is reflected in the results of several model systems in which GATA2 is required to maintain stem cell self-renewal capacity (Tsai & Orkin, 1997; Nottingham et al., 2007; de Pater et al., 2013). The absence of MLP/LMPP, loss of lymphoid and monocytedc hematopoiesis, with relative sparing of erythropoiesis and granulopoiesis, resembles an accelerated ageing phenotype (Gekas & Graf, 2013). It will be of interest to determine whether objective signs of this can be detected prematurely in GATA2 patients (Bakker & Passegave, 2013).

The parallel failure of monocytopenia and lymphopenia highlights the inadequacy of conventional models of haematopoiesis in which the primary bifurcation in cell fate is a split between ‘myeloid’ and lymphoid’. Conventionally, monocytes and lymphocytes would arise from opposite lineages. It is now thought that monocyte potential and indeed granulocyte potential is retained by primitive multi-lymphoid lymphoid or lymphoid-primed progenitors (Goardon et al., 2011; Doulatov et al., 2012) and the first fate decision is more accurately defined as the separation of megakaryocyte and platelet precursors.
erythroid potential from ‘nucleated cell potential’ (Arinobu et al, 2007; Sanjuan-Pla et al, 2013). Megakaryocytes are closely related to HSCs and the platelet lineage, including platelets themselves, retains a high level of GATA2 mRNA and GATA2 protein.

GATA2 mutation and immune dysfunction

The most common manifestations of immune dysfunction in GATA2 mutation are generalized warts and mycobacterial infection. Recurrent respiratory tract infection and a miscellany of infections due to impaired cell-mediated immunity including EBV, herpes simplex virus, varicella zoster virus (VZV), invasive aspergillus, histoplasmosis and candida are also seen (Vinh et al, 2010; Camargo et al, 2013; Dickinson et al, 2014; Spinner et al, 2014). The combination of impaired viral clearance and defective immunosurveillance is presumably responsible for a high rate of malignant transformation of HPV-driven neoplasia and increased incidence of solid neoplasms. In addition to direct infectious complications, pulmonary alveolar proteinosis may be exacerbated by recurrent respiratory and mycobacterial infections. It is difficult to tease out the precise mechanisms of immunodeficiency when so many components of innate and adaptive immune systems are compromised. Perhaps one of the most striking features of some patients with GATA2 mutation is how little infection they experience despite very profound cellular deficiencies. This suggests that, while immunocompetence is critical in childhood, immunological memory is the main factor sustaining adult resistance to infection.

Warts

It has been noted that few other conditions cause papillomatosis or generalized verrucosis to the same degree as GATA2 deficiency, namely Epidermodysplasia verruciformis (EV) warts, hypogammaglobulinemia, immunodeficiency, myelokathexis (WHIM) syndrome warts, immunodeficiency, lymphoedema and anogenital dysplasia (WILD) syndrome, DOCK8 deficiency syndrome and idiopathic CD4 lymphocytopenia (Vinh et al, 2010; West et al, 2014). These are easily distinguished on clinical grounds or routine investigation. The combination of warts with monocytopenia is highly suggestive of a diagnosis of GATA2 mutation.

Life without DCs

Lack of DCs was highlighted in the description of DCML deficiency (Bigley et al, 2011) but was also previously recorded in at least one case report (Witzke et al, 2004). The lack of DCs may impair recognition of viruses and intracellular pathogens contributing to disseminated herpes virus infection and mycobacterial susceptibility. In earlier case reports, defects in antigen-presenting cell-dependent mitogen responses (concanavalin A), responses to immunization, recall antigens and delayed type hypersensitivity are all documented (Kaur et al, 1972; Witzke et al, 2004). A systematic study of vaccine responses has not been performed. Interestingly, patients with undetectable blood (and probably also tissue DCs) still experience graft-versus-host disease when transplanted (Cuellar-Rodriguez et al, 2011). The persistence of tissue macrophages and epidermal Langerhans Cells may offer an alternative route of antigen presentation when DCs are profoundly depleted (Bigley et al, 2011).

Monocytes and mycobacterial infection

Excluding generalized BMF, GATA2 mutation and hairy cell leukaemia (HCL) are two conditions notable for monocytopenia. Monocytopenia in HCL is less severe than GATA2 deficiency but also known to confer a risk of mycobacterial infection of 4–9% (Thaker et al, 2001). Monocytopenia leads to a profound impairment of whole blood cytokine responses in tests used to screen for genetic susceptibility to mycobacterial infection. Both IL12 and γ-interferon (IFNγ) responses are blunted (Bigley et al, 2011). This might be a trivial result given the absence of monocytes, but can be argued that it is a useful reflection of the in vivo defect and how its magnitude compares with established molecular causes of mycobacterial susceptibility, such as IFNγ receptor mutations (Fischer, 2007). Resistance to mycobacterial infection is complex and the well-described IFNγ-IL12 axis is multiply compromised by the absence of NK cells and DCs, in addition to monocytes. Although tissue macrophages are present at sites of infection, organized granulomatous inflammation is deficient.

B cells

CD38⁺CD10⁺ B/NK precursors are not detectable in the BM (Bigley et al, 2011) and CD38⁺CD27⁻ transitional B cells, the most recent BM emigrants, are absent or severely depleted in GATA2 deficiency (Chou et al, 2014; Dickinson et al, 2014). Naïve B cells are also lower while there is a relative enrichment of memory B cells and plasmablasts. These changes are clearly consistent with failing production and the accumulation of differentiated cells. The NIH group first noted the presence of plasma cells and relatively normal immunoglobulin (Ig) in most patients (Vinh et al, 2010), although IgA deficiency and hypogammaglobulinemia presenting with recurrent sinusitis is reported (Chou et al, 2014). Plasma cells are found in the BM and in inflamed tissues, many with an abnormal CD56⁺CD19⁻phenotype (Calvo et al, 2011). GATA2 mutation quite powerfully demonstrates the autonomy of plasma cells in maintaining anamnestic humoral immune responses.
NK cells

The original report of human NK deficiency was a case of GATA2 mutation and other primary NK deficiency in humans is actually very rare (Biron et al, 1989; Mace et al, 2013). NK-mediated restriction of virally infected or dysplastic targets is impaired, weakening immunosurveillance of papillomatosis and other malignant transformations. The most obvious and consistent feature is loss of the CD56bright population of immature NK cells, analogous to transitional B cells (Mace et al, 2013; Dickinson et al, 2014). Remaining NK cells appear skewed toward a more mature phenotype with loss of NKG2A (KLRC1) and CD62L (SELL) and increased expression of killer cell immunoglobulin-like receptors (KIRs) (Dickinson et al, 2014). These features are quite variable between patients and may reflect viral infection history, although are not obviously connected to cytomegalovirus (CMV) serostatus. NK cells are known to contain some GATA2 mRNA and surviving cells appear to have additional functional defects that are not restored by γ-interferon (IFNγ) therapy in vivo (Mace et al, 2013). A few patients with absent NK precursors appear able to maintain relatively good total NK counts. The mechanism of this is unknown but may indicate the development of ‘NK memory’ in response to chronic antigen stimulation (Romee et al, 2012). Other innate lymphocytes, including CD161+ mucosal invariant T cells, are also depleted in GATA2 mutation, but NKT cells are less affected (Dickinson et al, 2014).

T cell abnormalities and premature immunosenescence

In contrast to the other lymphocyte subsets, T cells are relatively well-preserved in patients with GATA2 mutation. Peripheral T cell homeostasis in adults is largely independent of BM-derived T cell precursors. Thus in patients with progressive BMF, T cells, like plasma cells and Ig, can be maintained for many years. Inversion of the CD4:CD8 ratio (to <1) is a crude sign that CD4 helper function is failing and chronic antigen stimulation is driving expansion of CD8 memory cells (Vinh et al, 2010; Ostergaard et al, 2011; West et al, 2014). This is supported by more detailed phenotyping of T cell subsets showing diminution of naïve cells and expansion of terminally differentiated CD8+ CD45RA+ effector cells (TEMRA cells) with lower CD27, SELL (CD62L), CD38 and HLA-DR than healthy controls (Dickinson et al, 2014). Large granular lymphocytosis has been observed in GATA2 patients (Vinh et al, 2010; Spinner et al, 2014) and is the morphological correlate of TEMRA cell expansion (Clemenceau et al, 2008). Overall, the immunophenotype of GATA2 patients in all lymphoid compartments is strongly reminiscent of the pattern of terminal differentiation seen with advancing age and in chronic viral infections, such as CMV, hepatitis C and human immunodeficiency virus (Strindhäll et al, 2013). Thus, a prematurely aged phenotype is seen both in peripheral immune cells and in the BM progenitor compartment.

Macrophages, Langerhans cells and pulmonary alveolar proteinosis

The appearance of inflammatory macrophages in the absence of circulating monocytes was first noted by Vinh et al (2010). Macrophages were also reported in BM, lung and healthy skin of GATA2 patients (Bigley et al, 2011). In the epidermis, Langerhans cells survive, albeit in reduced numbers. Local proliferation of Langerhans cells is well-described, accounting for the independence of this population in mice (Merad et al, 2002). However, the presence of macrophages was more difficult to reconcile with conventional model of a mononuclear phagocyte system in which monocytes continuously give rise to tissue macrophages. Human HSC transplantation had already indicated that macrophages were considerably more stable than DCs (Haniffa et al, 2009) but full independence of macrophages from circulating monocytes was not anticipated. Models of tissue macrophage homeostasis have since been dramatically revised and their longevity and stability in the absence of monocytes is now well-established in murine experiments (Hashimoto et al, 2013).

Macrophage homeostasis is highly relevant to lung hygiene. Defects of alveolar macrophages lead to PAP through inadequate clearance of surfactant proteins (Carey & Trapnell, 2010). The primary cause of this rare condition is autoimmune antibodies to granulocyte-macrophage colony-stimulating factor (GM-CSF, CSF2), which impair the GM-CSF-mediated maturation of alveolar macrophages. Whole lung lavage and GM-CSF injection usually restores macrophage function in these cases. In contrast, patients with PAP due to GATA2 mutation do not have anti-GM-CSF antibodies and respond poorly to lavage and GM-CSF therapy. Although they have an adequate number of alveolar macrophages, both in biopsies and lavage fluid, a cell-intrinsic effect of GATA2 mutation appears to interfere with their functional maturation. GATA2 interacts with many signalling cascades through modulating the expression of key receptors or transduction proteins, such as the M-CSF (CSF1) receptor, phospholipase C and IL1 signalling pathway (Bigley et al, 2011; Ishijima et al, 2012; Wu et al, 2013). Protein-protein interactions are also reported with STAT proteins and SMAD (Ezoe et al, 2005; Dong et al, 2014) as well as direct effects on phagocytosis (Lasbury et al, 2003). The potential functions of GATA2 outside the nucleus are only recently recognized and further studies are required to elucidate the mechanism of PAP in GATA2 deficiency.

Thrombosis

GATA2 is highly expressed in megakaryocytes, platelets and vascular endothelium (Johnson et al, 2012; Lim et al, 2012)
and thrombosis is reported in up to 25% of patients (Spinner et al, 2014). Lupus anticoagulant, infection and malignancy add further to intrinsic risks and patients may develop refractory problems with thrombosis, cellulitis and soft tissue infections.

**Autoimmune manifestations**

Patients with GATA2 mutation experience a spectrum of autoimmune disease. Panniculitis, either as isolated inflammatory nodules or more classical erythema nodosum are possibly the most common but arthritis, lupus-like syndromes autoimmune hepatitis and primary biliary cirrhosis have also been described (Dickinson et al, 2014; Spinner et al, 2014). A severe deficit of Treg, was reported in DCML deficiency (Bigley et al, 2011). Although it could be argued that this was secondary to DC deficiency, a phenomenon reported in mice (Darrasse-Jeze et al, 2009), it is likely that widespread failure of mononuclear cell production is at least partly to blame in GATA2 mutation. In the B cell compartment, the CD38\(^{-}\)CD21\(^{+}\) population, previously reported to be associated with autoimmunity, is increased in some GATA2 patients (Dickinson et al, 2014).

**Solid malignancy**

Premature death has occurred in a number of patients due to solid malignancy. In keeping with the prevalence of HPV infection, anogenital intraepithelial dysplasia is a serious concern and squamous cell carcinoma occurs with higher frequency than expected (Dickinson et al, 2014; Spinner et al, 2014). EBV-related mesenchymal tumours, adenocarcinoma, desmoid tumour of the chest wall and schwannoma have also been reported (Dickinson et al, 2014; Spinner et al, 2014).

**Lymphedema, deafness, congenital anomalies and preterm labour**

Lymphedema and myelodysplasia are the primary features of Emberger syndrome (Ostergaard et al, 2011). Deafness is also seen especially in Emberger syndrome and occurs with whole gene deletions of GATA2 (Ostergaard et al, 2011; Kazenwadel et al, 2012) possibly due to failure of generation of the perilymphatic space surrounding the semi-circular canals (Haugas et al, 2010). The evolution of lymphoedema is puzzling. Unlike most congenital forms of lymphoedema, which occur bilaterally as a child begins to walk, patients with GATA2 mutation often describe a precipitating event occurring later and leading to unilateral limb swelling. It has been elegantly demonstrated that GATA2 is expressed in endothelial cells and lymphatic valves (Kazenwadel et al, 2012; Lim et al, 2012) but it is also conceivable that the BM-dependent development or maintenance of lymphoid tissue is additionally culpable. Suggestions that N-terminal frameshift mutations or larger deletions of GATA2 are more likely to cause lymphoedema and non-haematopoietic defects are supported by the larger cohort studies (Spinner et al, 2014).

As initially noted by Vinh et al (2010), premature labour is often experienced by females with GATA2 mutation (Dickinson et al, 2014; Spinner et al, 2014). The problem appears to be maternal rather than fetal as it affects wild-type fetuses of mothers with mutations but not fetuses with de novo mutations. There are many potential explanations for premature labour including roles for GATA2 in the uterus and placenta (Ma et al, 1997; Rubel et al, 2012). In a single case, successful BM transplantation allowed the patient to carry a second pregnancy to term (unpublished observations).

**Acquired genetic abnormalities and evolution to leukaemia**

Hereditary MDS/AML, rather than immune dysfunction, is the principle clinical feature of several kindreds with GATA2 mutation (Hahn et al, 2011; Bodor et al, 2012; Holme et al, 2012; Ishida et al, 2012; Fujitara et al, 2014). MDS/AML is

| GATA2 configuration | Associated with | Outcome |
|---------------------|-----------------|---------|
| Germine heterozygous mutation | ASXL1 monosomy 7 | High risk MDS/AML |
| Somatic heterozygous mutation (often ZF1; also ZF2) | bi-CEBPA m-CEBPA t(15;17) NPM1 RUNX1, IKZF1, NRRAS, KRAS, FLT3-ITD, KIT, WT1, FBX03, MLF1P, STT3B, IDH1, DNMT3A | Favourable risk AML |
| Somatic heterozygous mutation (often ZF2) | t(9;11) Philadelphia ANO5, MAX, ENO1, COL3A1, AFP, SERPINA1, MGAT5B, ZNF208 | Intermediate-high risk AML |
| Transposition of GATA2 distal enhancer (G2DHE; −110 kb) | inv(3)(t;3;3) Activation of EVI1 by G2DHE monosomy 7 | High risk MDS/AML |

ZF, zinc finger; G2DHE, GATA2 distal haematopoietic enhancer; bi, bi-allelic; m, mono-allelic; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome. Bold in column 2 represents the mutations most commonly associated with the corresponding GATA2 mutation in column 1.
the presenting feature of 30–50% of cases and the actuarial risk of developing MDS/AML by 60 years of age is close to 90% (Dickinson et al, 2014; Micol & Abdel-Wahab, 2014; Spinner et al, 2014). Initially, it was thought that point mutation of the second zinc finger, such as T354M, might confer an increased risk of leukaemic transformation over frameshift mutations or null alleles but this is not borne out by larger cohort studies (Dickinson et al, 2014; Spinner et al, 2014). Constitutive genetic background may have an influence on the risk of leukaemic transformation and susceptibility to infection although it is notable that a range of clinical phenotypes can be seen in different individuals within one pedigree (Holme et al, 2012; Mutsaers et al, 2013; Spinner et al, 2014). The acquisition of additional genetic abnormalities in the transformation of GATA2 mutation to multilineage dysplasia is clearly presaged by the high incidence of monosomy 7 and trisomy 8 in familial cases of MDS/AML (Hahn et al, 2011; Ostergaard et al, 2011; Bodor et al, 2012; West et al, 2013; Dickinson et al, 2014; Micol & Abdel-Wahab, 2014; Spinner et al, 2014). Recently, acquired mutation of ASXL1 (chr 20q11) has been demonstrated in approximately 30% of individuals with GATA2 mutation evolving to MDS. Acquired ASXL1 mutation is strongly associated with the presence of monosomy 7, BM hypercellularity and chronic monomyelocytic leukaemia (Bodor et al, 2012; West et al, 2013; Micol & Abdel-Wahab, 2014). Whole genome sequencing in one patient has also identified mutations in EZH2, HECW2 and GATA1 (Fujiiwara et al, 2014); the spectrum of somatic mutations that are known to occur with germline GATA2 mutation is summarized in Tables IV and SI. The presence of monosomy 7, ASXL1 mutation and trilineage dysplasia are all high risk features in the biogenesis of AML (West et al, 2013). A number of patients with GATA2 mutation have received successful haematopoietic stem cell transplantation, precisely because a high risk AML was detected, according to standard criteria. The detection of a GATA2 germ-line mutation does not appear to mitigate the risk of AML that follows, whatever the subsequent genetic events (Cuellar-Rodriguez et al, 2011; Dickinson et al, 2014; Spinner et al, 2014).

The knowledge that GATA2 mutation is a constitutive risk factor for MDS/AML begs an important question of whether acquired GATA2 mutation is among the key leukaemia-initiating events in sporadic MDS/AML (Table IV). The incidence in unselected cases of MDS or AML is actually quite low, at <5%, and may include cases of GATA2 germ-line mutation that were assumed to be somatic, in the absence of a germ-line DNA control (Yan et al, 2011; Luesink et al, 2012; Papaemmanuil et al, 2013; Shiba et al, 2014). Gain of function mutation L359V has been documented in blast transformation of CML, associated with typically poor outlook (Zhang et al, 2008, 2009). In contrast, a high level of mutation (approximately 40%) is observed with bi-allelic mutation of CEBPA, conferring a better prognosis than CEBPA mutation with wild-type GATA2 (Greif et al, 2012; Fasan et al, 2013; Green et al, 2013; Grossmann et al, 2013; Shiba et al, 2014). Excluding those cases with FLT3-internal tandem duplication (ITD) (which associates with GATA2 wild-type) may lessen the observed beneficial effect of a GATA2 mutation (Green et al, 2013).

Although convincing evidence of recurrent mutation of GATA2 was not originally found in sporadic AML, it was first noted more than 10 years ago, that the distal 5’ regulatory elements of GATA2, were involved in chromosome 3q21 rearrangements and that dysregulation of GATA2 expression might contribute to leukaemogenesis (Zhou et al, 1998; Wieser et al, 2000). This hypothesis has recently been elegantly confirmed, with the demonstration that 3q rearrangement is a double hit disease involving simultaneous removal of the GATA2 distal haematopoietic enhancer (G2DHE) and cis-activation of the neighbouring locus EVI1 (Groschel et al, 2014; Yamazaki et al, 2014). Thus loss of GATA2 expression is closely involved in leukaemogenesis involving 3q rearrangements. The risk profile of MDS/AML associated with GATA2 mutation thus depends upon the germline configuration of GATA2 and the sequence and location of associated genetic events (Table IV). Somatic monosomy 7 confers a high risk phenotype upon MDS/AML arising with germline

| Table V. Investigation of GATA2 deficiency. |
|--------------------------------------------|
| **History and examination**                |
| Blood count: Monocytopenia (may be obscured by progenitor mobilization or atypical lymphocytosis during infection) |
| Lymphocyte subsets: B cell and NK cell deficiency; CD4:CD8 inversion (<1:0); absence of CD1c<sup>+</sup>, CD14I<sup>+</sup> and plasmacytoid blood DCs |
| Immunoglobulins normal levels, occasional IgA or IgG deficiency |
| Bone marrow: megakaryocyte dysplasia, hypopcellularity, fibrosis; cytogenetics: monosomy 7; trisomy 8; ASXL1 mutation |
| Further investigations                     |
| Blood: Lupus anti-coagulant; elevated FLT3 ligand (10- to 100-fold); absent transitional B cells and CD56<sup>bright</sup> NK cells |
| Bone marrow Flow cytometry: loss of primitive MPL, LMPP population and reduction of GMP; CD56<sup>+</sup> plasma cells present |
| Lungs: diminished lung volumes and transfer factor; pulmonary infiltrates on CT; pulmonary alveolar proteinosis on biopsy without GM-CSF antibodies |
| Tissues biopsies: special stains for mycobacteria and fungi; neoplastic lesions investigated for HPV and herpes virus nucleic acid or antigens |
| Confirmatory test                            |
| GATA-2 gene deletion, mutation in codons 1–398 or intron 5 enhancer |

MLP, multi-lymphoid progenitors; LMPP, lymphoid-primed multi-potent progenitors; GMP, granulocytic mononcytic progenitors; CT computerized tomography.
GATA2 mutation, while somatic mutation of GATA2 with pre-existing bi-allelic CEBPA mutation is favourable risk. Other pre-existing mutations are intermediate or high risk (e.g. FLT3-ITD, RAS mutations and WT1 mutation). The double jeopardy of somatic inv3 or t(3;3) in reducing GATA2 but co-activating EVI1 is undoubtedly high risk.

Clinical management of individuals with GATA2 mutation

Recognition of the clinical syndromes of GATA2 deficiency and genetic diagnosis have facilitated the rapid identification of more than 200 individuals with GATA2 mutation (Dickinson et al., 2014; Spinner et al., 2014). Cross-sectional analysis of these is beginning to inform clinical management. A continuing challenge is the recognition of new cases that may present with a spectrum of manifestations to haematologists, infectious disease physicians, dermatologists, chest physicians, rheumatologists and many other specialties. Monocytopenia remains the most vital clue to the haematologist making a remote diagnosis. Suggested follow up investigations are listed in Table V.

With family members being diagnosed through genetic testing, it is important that this is performed with appropriate counselling. GATA2 deficiency and its definitive treatment are significant health issues. An additional complication is the pressure upon siblings to be screened as potential HSC donors. Their right to refuse genetic screening needs to be considered prior to tissue typing to prevent undue coercion.

With a median survival of 60 years (Spinner et al., 2014), a watch and wait policy is acceptable for many patients. Warts vary in severity but can be difficult to treat. It is prudent to treat respiratory infection promptly and to maintain vigilance for mycobacterial infection. Patients will benefit from serial pulmonary function testing, computerized tomography scanning and, possibly, prophylactic azithromycin. Panniculitis can be painful and responds to steroids, although steroid-sparing agents, such as dapsone, are preferable. The use of corticosteroids is difficult as blood counts, chest symptoms and inflammatory problems may respond promptly but caution is advisable in view of the level of subclinical immunopa-

Serial monitoring of peripheral blood leucocytes is useful and annual BM examination for morphology, flow cytometry and cytogenetics is advisable. FLT3 ligand, although not a routine test, shows a good correlation with clinical and cellular progression. ASXL1 mutation screening of blood or BM samples is likely to be highly informative for the risk of leukaemic transformation.

Haematopoietic stem cell transplantation has been performed on at least 30 patients worldwide and has a good outcome (Cuellar-Rodriguez et al., 2011; Spinner et al., 2014). The development of MDS or AML, often with high risk cytogenetic features, has been an automatic trigger for many patients (most of whom were diagnosed with GATA2 mutation retrospectively). The decision to move to transplant should not be delayed if cytogenetic abnormalities arise or there is a life-threatening complication at any stage. Although quite advanced PAP responds impressively (Cuellar-Rodriguez et al., 2011), salvaging patients in this situation is not an ideal strategy that should be obviated with genetic diagnosis and prospective monitoring. HPV infection responds well to transplantation and reversal of stage III intra-epithelial neoplasia, obviating potentially disfiguring surgery, has occurred over 12–18 months, possibly aided by HPV vaccination (unpublished observations). Close liaison with other specialist services and vigilance for monocytopenia will always be required to avoid the inadvertent progression of patients with unusual presentations, beyond the hope of cure. In the future, familial GATA2 mutation may be an ideal opportunity to test gene therapy strategies as it is predicted that correction of haplo-insufficiency would lead to a competitive survival advantage for HSC at the BM niche.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table SI. Reported mutations of GATA2 in humans.

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