Clinical data

Patient 1 (FHCRC-97)

Patient 1 was healthy until age 10 years, when she presented with recurrent febrile illnesses. During one such episode she was found to have respiratory infection with parainfluenza virus and mycoplasma. At this time she was found to have pancytopenia with a white blood cell count of $2.3x10^9$/L, hemoglobin 94 g/L, hematocrit 26.7%, MCV 92.4 fL, and platelet count $72x10^9$/L. Differential peripheral blood cell count showed 69% neutrophils, 25% lymphocytes, 4% monocytes, 1% eosinophils. Reticulocyte count was low at 0.4. IgG, IgM, and IgA were normal, and C3 and C4 were normal. On blood smear, no schistocytes, spherocytic predominance, nucleated red cells, nor teardrop cells were noted. Bone marrow aspiration showed 10-15% overall cellularity with megakaryocytes present, progressive myeloid and erythroid maturation, and no evidence of leukemia or tumor infiltration. Cytogenetics was positive for deletion of 5q by FISH and by G-band karyotypes (46,XX,der(1)(qter→q?12::p?31→qter),der(5)t(1;5)(p?31;q2?2)[19] / 46,XX[1]). Additional marrow testing revealed no mutations in *TERC, DKC1* or *SBDS*. Red cell Adenosine Deaminase was normal. Telomere length was not suggestive of dyskeratosis congenita. Blood cell counts continued to remain low after resolution of her infections. HLA-B allele mismatched unrelated donor bone marrow transplant was conducted 4 months later with busulfan/cyclophosphamide conditioning. Patient developed severe acute GVHD of skin and gut, treated with mesenchymal stem cells, extracorporeal photochemotherapy, mycophenolate mofetil, corticosteroids, FK506. After 1 year post transplant, marrow showed no evidence of disease, with normal cytogenetics and 100% donor cells. GVHD was asymptomatic and minimal. This patient has no history of limb swelling or lymphedema.

Patient 2 (FHCRC-84)

Patient 2 is the older sister of Patient 1. At age 14 years, she was evaluated as a potential HSC donor for her sister. Bone marrow aspiration with karyotype and FISH were performed to rule out familial 5q- syndrome and proved negative but did reveal trilineage dyspoiesis. Peripheral blood counts showed platelets of 140 $x10^9$/L and an absolute neutrophil count $1.6x10^9$/L. By age 17, platelet count was $105x10^9$/L, ANC was 1.3 $x10^9$/L, and hemoglobin was 1.33 g/L, with WBC of 5.0 $x10^9$/L and differential count showing 26.8% neutrophils, 62.1% lymphocytes, 10.1% monocytes, 0.8% eosinophils, and 0% basophils. Bone marrow evaluation 3 months later revealed normal morphology with 1/20 cells exhibiting monosomy 7 by routine cytogenetics and, employing FISH, monosomy 7 was detected in 11/200 (6%) cells. Bone marrow repeated again 3 months later

showed 1/20 monosomy 7 cells by routine cytogenetics and 5% monosomy 7 by FISH, indicating stable disease. Telomere lengths were low in the granulocyte subset but normal in lymphocyte subsets. Marrow fibroblast DNA from this patient was used to confirm the germline nature of the mutation in this family. She had no history of recurrent infections. She is currently clinically stable and has no history of limb swelling or lymphedema.

Patient 3 (FHCRC-95)

Patient 3 is the mother of patients 1 and 2. She has a history of low blood counts since age 15-16 years with a white blood cell count running between approximately 3-4 $x10^9$/L, hematocrit 33-34%, and platelet count of about $100x10^9$/L. As a child, she had a history of recurrent pneumonias. Marrow results at age 44 years showed trisomy 8 by FISH. Her maternal grandmother developed AML in her 50s. Patient 3 has no history of limb swelling or lymphedema.

Patient 4 (GC54819)

Patient 4 presented at age 14 years with an infection that was not resolving. He had a white blood cell count of 1.6-2.6$x10^9$/L, haemoglobin of 63 g/L and platelets that fell to $39x10^9$/L. A bone marrow examination did not reveal a cause for the pancytopenia, but showed a hypocellular marrow with dysplastic megakaryopoiesis. Karyotype analysis revealed a deletion at chromosome 3q21.3q-q22.2, and microarray analysis confirmed the deletion, further specifying it to be an 8.9 Mb deletion of 3q21.3-3q22.3 (minimum deletion chr3:127,966,423-136,853,218, hg19; maximum chr3:127,927,712-136,889,323). He had no history of lymphedema or swelling.

Patient 4 was the child of an 18 year old G1P0 (first pregnancy) woman, in which this pregnancy was complicated by gestational diabetes (controlled by diet) and a urinary tract infection. There was exposure to smoking and the oral contraceptive pill until 3 months gestation. Patient 4 was born at 38 weeks gestation by spontaneous vaginal delivery. His birth weight was 6 pounds, 15 ounces. He was in hospital for 2 weeks post delivery on account of some initial respiratory and feeding problems. From birth, he was noted to have dysmorphic features. He had significantly delayed development of a moderate to severe nature.

Patient 4 was initially seen at one month of age. His length was 53.5 cm (25-50^{th} centile), his weight was 3.7 kg (25^{th} centile) and his head circumference was 35.7 cm (10-25^{th} centile). His face was dysmorphic with downward slanting palpebral fissures and marked bilateral ptosis with the right side more affected than the left. There was a small hemangioma of the left eyelid. He had anteverted nares. His ears were posteriorly rotated. He had a highly arched palate. He had

micrognathia. He had mild syndactyly of his hands with a transitional crease of the left palm. His hand measurements were normal (approximately 50th centile). He had a small umbilical hernia and glandular hypospadias and both testes were descended. He had hypotonia. He also had blocked tear ducts bilaterally as an infant. He had an excessive gag. Cranial nerves were normal. The tone in his upper extremities was slightly reduced as was his truncal tone while that of the lower extremities was increased. He had global hyperreflexia but normal sensation and apparently normal coordination. The plantar responses were difficult to assess as he had a lot of withdrawal. He developed torticollis as an infant. He developed failure to thrive and by 34 months; his weight was 12.125 kg (5th centile), height was 90.1 cm (5th centile) and his head circumference was 49.8 cm (25-50th centile). He developed a hip dislocation in childhood. He had severe constipation.

Patient 4 was next seen at 15 years of age. His hair was normal. He had an unusual right eyebrow. He had bilateral ptosis. He had a high bridge of his nose. His alae nasi were narrow. He had a short, well-formed philtrum. His upper lip was prominent and thicker than the lower lip. He had crowded teeth and a highly arched palate. His right superior pinna was large and thin and his left was less so with better folding over of the pinna. There was some freckling of his face near the lateral part of his right eye and he also had a darker line on his abdomen which was consistent with mild hyperpigmentation. His hands were thin and he had arachnodactyly and he had wasted thenar muscles. His legs had muscle wasting especially distally and he had progressive talipes.

Investigations revealed mild hydronephrosis of his right kidney. An echocardiogram was normal. A CT scan of his head revealed generalized atrophy with complete agenesis of the corpus callosum. Additionally, there was the impression of a small bony defect in the planum sphenoidale. An abdominal ultrasound in 1996 revealed severe dilatation of the right intrarenal collecting system and right proximal ureter. There was no discernible right renal parenchyma identified. The left kidney appeared normal as did the left intrarenal collecting system.

Patient 5

Patient 5 was previously described by Callier *et al.*[1]. She was diagnosed at birth with dysmorphic craniofacial and other body features. She displayed developmental delay, partial agenesis of the corpus callosum and was severely mentally retarded. At 11 years of age, she developed pancytopenia with MDS which was associated with gingivitis and staphylococcal skin infections. CGH and FISH analyses identified an interstitial 6.9 Mb 3q21.1-q21.3 deletion encompassing genes *ADCY5* to *TRH* (~chr3:123,000,000-129,700,000).

Patient 6 (GC42542)

Patient 6 was born at 31 weeks gestation with associated complications due to prematurity. He displayed dysmorphic craniofacial features, seizures (onset at 6 years) and delayed intellectual development. At 6.5 years, primary lymphedema was evident in both legs and progressed to include the scrotum by age 16. At 10.5 years, he presented with recurrent infections and low neutrophil and platelet counts. Serial bone marrow aspirations revealed trilineage dysplastic changes. MDS was diagnosed at age 16 years and complicated by persistent anemia and refractory cytopenias by age 20 years. At 29 years, his disease progressed to AML and he died from this disease at age 30. The combination of lymphedema with MDS/AML resembles Emberger syndrome (OMIM 614038). CGH microarrays of skin, peripheral blood and bone marrow identified a constitutional 8.2 Mb deletion of chr3:120,247,726-128,319,968 (minimum; hg19 coordinates), chr3:120,154,188-128,324,987 (maximum) encompassing numerous genes including *GATA2*. All bone marrow cells were trisomy 21 by age 27 years.

Patient 7

Patient 7 is a 20 year old Caucasian male from Canada with a history of severe lymphedema of both lower legs since birth who presented at the age of 14 with pancytopenia. He was found to have a hypocellular bone marrow with monosomy 7 by cytogenetics. At the age of 18 he was diagnosed with myelodysplastic syndrome with a hypercellular bone marrow and monosomy 7 and trisomy 8 by cytogenetics. He was treated with two cycles of 5-azacytidine. However, he progressed to acute myelogenous leukemia and received three cycles of daunorubicin and cytarabine. Despite an initial remission for two months, he relapsed with leukemia. He received two cycles of induction chemotherapy- one cycle with Clofarabine, Idarubicin, and cytarabine and one cycle with mitoxanthone and etoposide. The presence of persistent blasts on repeat bone marrow examination led to a myeloablative transplant: conditioning with cyclophosphamide 120 mg/kg and 12 Gy total body irradiation followed by a 10/10 matched unrelated donor peripheral blood stem cell transplant at age 18 years. His post-transplant course was complicated by mild graft-versus-host-disease of the gastrointestinal tract. One year post-transplant he had no evidence of leukemia on bone marrow biopsy. However, he returned eighteen months post-transplant with bilateral knee pain and back pain. He was found to have 13 percent blasts in the peripheral blood, and 17 per cent blasts in the bone marrow biopsy. He is currently being re-induced with high dose cytarabine in anticipation of a second unrelated donor transplant.

Patient 8 (13.I.2)

Patient 8 was previously described by Vinh *et al.*[2] and Hsu *et al.*[3]. She was found to have monocytopenia, B and NK cell lymphopenia, and warts. Together with her son's (Patient 9) symptoms, this is indicative of familial MonoMAC syndrome. She also suffered from unilateral lymphedema (left), and again, together with the MDS present in her son, this implicated Emberger syndrome. She is otherwise healthy. Recent genetic analysis identified an incomplete deletion of the *GATA2* gene (NM_032638.4), c.1-200_871+527del 2033 bp (p.Met1del290) resulting in what would be predicted to be a null mutation.

Patient 9 (13.II.1)

Patient 9 was previously described by Vinh *et al.*[2] and Hsu *et al.*[3]. He is the son of Patient 8 and had an undefined immunodeficiency. At 33 years of age, he presented with fever, disseminated histoplasmosis and pancytopenia. Further investigation revealed disseminated *Mycobacterium avium* complex (MAC). Together this is indicative of MonoMAC syndrome. Bone marrow analyses showed MDS with monosomy 7. He contracted and was treated for disseminated *N. udagawae* infection, was treated for brain edema, and succumbed at age 34 years. While he showed no signs of lymphedema, his mother (Patient 8) suffered from lymphedema, which together with his MDS suggested familial Emberger syndrome. Like his mother, he demonstrated the same deletion in the *GATA2* gene (NM_032638.4), c.1-200_871+527del 2033 bp (p.Met1del290) confirming germline transmission.

Patient 10

Patient 10 is a 20 year old Caucasian male from Australia who was first diagnosed with myelodysplasia at age 19 years. He presented with severe acne for consideration of Isotretinoin therapy. On examination he was noticed to have widespread warts on his upper and lower limbs in addition to severe acne. He had no family history of MDS/AML, recurrent infections or lymphedema. Screening by complete blood examination revealed neutropenia (1.18×10^9/L) and monocytopenia (0.01×10^9/L). Bone marrow biopsy revealed trilineage dysplasia with 13% blasts (refractory anemia with excess blasts-2 (RAEB-2)) and cytogenetic analysis revealed monosomy 7.

Homology modeling of GATA2 WT and mutants

WT GATA2 was aligned with the template structure of murine Gata3 whose structure was obtained by X-ray crystallography[4]. Template alignment indicates that GATA2 is 97% (56/58) similar to murine Gata3. Subsequently, the zinc finger 2 motif was subjected to homology modeling. The alignment mode of Swiss model workspace (http://swissmodel.expasy.org/)[5] was used to predict the theoretical 3 dimensional structure of WT GATA2. For modeling of the mutants, WT residues were replaced with disease associated residues *in silico* by modifying the input primary sequences. The models were analyzed, fine tuned and opportunely minimized using the Swiss PDB viewer. The final results were further verified using the online tools PROCHECK, WHAT_CHECK, ERRAT VERIFY 3D and PROVE on the Structure Analysis and verification Server at http://nihserver.mbi.ucla.edu/SAVES/. The final structures were presented with PyMOL 0.99rcs6 (Innocentive Product, Delano Scientific LLC.).

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Table S1. ***Primers used for real-time RT-PCR analysis***

Gene	Forward Primer	Reverse Primer
Actb	5'-GATCATTGCTCCTCCTGAGC-3'	5'-GTCATAGTCCGCCTAGAAGCAT-3'
Angpt2	5'-GATCTTCCTCCAGCCCCTAC -3'	5'-CAGCAAGCTGGTTCCAATCT-3'
Gata2	5'-ATGGGCACCCAGCCTGCAAC-3'	5'-GTGGCCCGTGCCATCTCGTC -3'
Itga9	5'-GCCAGGCCGGAATAGCAGGC-3'	5'-GTGGCCAGCCGTCACTGCAT-3'
Kdr	5'-TGGAAACAGAATTTCCTGGG-3'	5'-ATAGCTCAATTTCATGCGGG-3'
Nfatc1	5'-CGAGTTCGATCAGAGCGGCGGG -	5'-ATGCTGCTGGCAAGGCAGAGTG -3
Pecam1	5'-AACAGAAACCCGTGGAGATG-3'	5'-GTCTCTGTGGCTCTCGTTCC-3'
Prox1	5'-CTGGGCCAATTATCACCAGT-3'	5'-GCCATCTTCAAAAGCTCGTC-3'

Patient ID	Number of patients	Familial or *de novo*	GATA2 Mutation	Genomic, cDNA and protein change	GATA2 Function	Overall Phenotype	Lymphedema (age onset - years)	Hematological abnormalities (age onset - years) with acquired chromosomal anomolies	Immunodeficiency/Infections (age onset - years)	Publications
Patient 1 (FHCRC-97)	1 Female (daughter)	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A, ID	None	MDS with 5q- clone, aplastic anemia (10)	Recurrent febrile illness, parainfluenza, mycoplasma	This publication
Patient 2 (FHCRC-84)	1 Female (daughter)	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A	None	MDS (14) with 5-6% monosomy 7, thrombocytopenia (14)	No	This publication
Patient 3 (FHCRC-95)	1 Female (mother)	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A, ID	None	MDS (44) with trisomy 8, leukopenia, anemia, thrombocytopenia (15)	Recurrent infections (pneumonias) as a child	This publication
Patient 10	1 Male	*de novo*	p.?¶	chr3:g.128,202,701A>C *GATA2* c.1017+2T>G (p.?)	LOF ZF(-)	M/A, ID	None	MDS (19), monocytopenia (19)	Warts, severe acne	This publication
Patient 4 (GC54819)	1 Male	*de novo*	Contiguous gene deletion encompassing GATA2 gene	chr3:g.127,966,423-136,853,218 (Min) chr3:g.127,927,712-136,889,323 (Max) (8.9 Mb 3q21.3-q22.3 deletion)	Null	M/A, ID, DF, NS	None	MDS (dysplastic megakaryopoiesis), leukopenia, anemia, thrombocytopenia, dysplastic megakaryopoiesis	Infections associated with pancytopenia	This publication
Patient 5 (French girl)	1 Female	*de novo*	Contiguous gene deletion encompassing GATA2 gene	6.9 Mb interstitial 3q21.1-q21.3 deletion (genes ADCY5 - TRH) (~chr3:g.123,000,000-129,700,000)	Null	M/A, ID, DF, NS	None	MDS (11) with monosomy 7 on 7/22 metaphases, pancytopenia (11)	Gingivitis & staphylococcal skin infections	This publication and Callier 2009 (Sref 1)
Patient 6 (GC42542)	1 Male	*de novo*	Contiguous gene deletion encompassing GATA2 gene	chr3:g.120,247,726-128,319,968 (Min) chr3:g.120,154,188-128,324,987 (Max) (8.2 Mb 3q13.33-q21.3 deletion)	Null	L, M/A, ID, DF, NS	Lymphedema (6.5) (bilateral)	MDS/AML (16/29) with trisomy 21	Recurrent pneumonia and ear infections, *H. influenza* meningitis	This publication and Wildin submitted
Patient 7	1 Male	*de novo*	p.Leu332Thrfs*53	chr3:g.128,202,727-128,202,728insGGTC *GATA2* c.992_993insGACC (p.Leu332Thrfs*53)	LOF ZF(-)	L, M/A	Lymphedema (birth) (bilateral)	pancytopenia (14) with monosomy 7, MDS (18) with monosomy 7 and trisomy 8, AML (18)	No	This publication
Emb-01	2 Males, 3 Females	Familial	p.Leu105Profs*15	chr3:g.128,205,129-128,205,130dupGG *GATA2* c.312_313dupCC (p.Leu105Profs*15)	LOF ZF(-)	L, M/A, ID	Lymphedema (birth-14, 44§) (3 bilateral, 1 unilateral left)	MDS (9,11,50), AML (9,11,53) two with monosomy 7, low CD4/CD8 ratio	Cutaneous warts with malignant transformation to anogenital dysplasia, unilateral ptosis	Ostergaard 2011 (Sref 6)
Emb-02	1 Males, 2 Females	Familial	p.Arg78Profs*107	chr3:g.128,205,211dupG GATA2 c.230-1_230insC (p.Arg78Profs*107)	LOF ZF(-)	L, M/A	Lymphedema (16) (1 bilateral, 2 unilateral left)	MDS (17), AML (17) with monosomy 7, immature bone marrow with monosomy 7	None	Ostergaard 2011 (Sref 6)
Emb-03	1 Female	*de novo*	p.Arg337*	chr3:g.128,202,711G>A *GATA2* c.1009C>T(p.Arg337*)	LOF	L, M/A	Lymphedema (6) (bilateral)	MDS (12), AML (12) with monosomy 7	None	Ostergaard 2011 (Sref 6)
Emb-04	1 Male	*de novo*	p.Ala341Profs*45	chr3:g.128,200,783-128,200,786del *GATA2* c.1019_1022delCGGC (p.Ala341Profs*45)	LOF ZF(-)	L, M/A, HL	Lymphedema (6) (unilateral left)	MDS (11)	None	Ostergaard 2011 (Sref 6)
Emb-05	1 Female	*de novo*	p.Ala341Argfs*38¶	chr3:g.128,200,774-128,200,790del *GATA2* c.1018-3_1031del17 (p.Ala341Argfs*38)¶	LOF ZF(-)	L, M/A, ID, HL	Lymphedema (birth) (bilateral)	Low CD4/CD8 ratio	Cutaneous warts with malignant transformation to anogenital dysplasia	Ostergaard 2011 (Sref 6)
Emb-06	1 Male	*de novo*	p.Cys373Arg	chr3:g.128,200,688A>G *GATA2* c.1117T>C (p.Cys373Arg)	LOF	L, M/A, ID	Lymphedema (8) (bilateral)	MDS (16) with monosomy 7	Warts	Ostergaard 2011 (Sref 6)
Emb-07	1 Male	*de novo*	p.Arg361Leu	chr3:g.128,200,723C>A *GATA2* c.1082G>T (p.Arg361Leu)	DB(-) * SM	L, M/A, ID, HL	Lymphedema (10) (bilateral)	Low CD4/CD8 ratio	Cutaneous warts with malignant transformation to anogenital dysplasia	Ostergaard 2011 (Sref 6)
Emb-08	1 Male	*de novo*	p.Ala194Serfs*8	chr3:g.128,204,862dupT *GATA2* c.579_580insA or c.579dupA (p.Ala194Serfs*8)	LOF ZF(-)	L, M/A	Lymphedema (8) (unilateral right)	AML (12) with monosomy 7	None	Ostergaard 2011 (Sref 6)
Kindred 13.I.2 (Patient 8)	1 Female (mother)	Familial	p.Met1del290	chr3:g.128,204,043-128,211,896 *GATA2* c.1-200_871+527del 2033 bp (p.Met1del290)	Null	L, ID	Lymphedema (unilateral left)	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia	Warts (adulthood), no history of infections	This publication, Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 13.II.1 (Patient 9)	1 Male (son)	Familial	p.Met1del290	chr3:g.128,204,043-128,211,896 *GATA2* c.1-200_871+527del 2033 bp (p.Met1del290)	Null	M/A	None	MDS (33) with monosomy 7 on all metaphases, trisomy 8, pancytopenia (33)	disseminated MAC, fungal infections, warts, recurrent pneumonias	This publication, Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 1	3	Familial	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *	M/A like, ID	None	Chronic myelomonocytic leukemia	HPV, MAC and various infections	Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 2	1	Familial	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *	M/A, ID	None	MDS-RE, thrombocytopenia	MAC	Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 3	1	S	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 9	1	S	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 21	1	?	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *		None			Hsu 2011 (Sref 3)
Kindred 5	1	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A, ID	None	MDS	*M. tuberculosis* , MAC, Warts, *M. abscesus* and HPV infeaction	Vinh 2010, Hsu 2011 (Srefs 2,3)

Kindred 17	1	?	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #		None			Hsu 2011 (Sref 3)
Kindred 19	1	?	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	ID	None		*M. massiliense* and chronic EBV infection	Hsu 2011 (Sref 3)
Kindred 8	1	*de novo*	p.Gly82Argfs*10	chr3:g.128,205,198delTinsGC *GATA2* c.243_244delAinsGC (p.Gly82Argfs*103)	LOF ZF(-)	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 10	1	*de novo*	p.Asn371Lys	chr3:g.128,200,692G>C *GATA2* c.1113 C>G (p.Asn371Lys)	DB(-) * SM	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 12	1	*de novo*	p.Arg361_Asn364del	chr3:g.128,200,711_128,200,722del *GATA2* c.1083_1094del (p.Arg361_Asn364delRNAN)	LOF ZF(-)	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 15	1	*de novo*	p.Arg396Trp	chr3:g.128,200,119G>A *GATA2* c.1186C>T (p.Arg396Trp)	DB(-) * SM	ID	None	Monocytopenia & B-cell, NK-cell and T-cell lymphocytopenia		Vinh 2010, Hsu 2011 (Srefs 2,3)
Kindred 18	1	?	p.Arg396Gln	chr3:g.128,200,118C>T *GATA2* c.1187 G>A (p.Arg396Gln)	LOF SM		None			Hsu 2011 (Sref 3)
Kindred 20	1	?	p.Asp259fs*	chr3:g.128,204,662_128,204,663ins 10bp *GATA2* c.778_779ins 10bp (p.Asp259fs*)	LOF ZF(-)		None			Hsu 2011 (Sref 3)
Kindred 22	1	?	p.Asn317fs*	chr3:g.128,202,768_128,202,769ins 11bp *GATA2* c.951_952ins 11bp (p.Asn317fs*)	LOF ZF(-)		None			Hsu 2011 (Sref 3)
Kindred 23	1	?	p.Pro254Leu	chr3:g.128,204,729G>A *GATA2* c.761C>T (p.Pro254Leu)			None			Hsu 2011 (Sref 3)
Kindred 24	1	?	p.Ser340_Asn381del	chr3:g.128,200,788C>T *GATA2* c.1018-1G>A (p.Ser340_Asn381del)	LOF ZF(-)		None			Hsu 2011 (Sref 3)
Subject 1	1	*de novo*	p.Gly200Glyfs*1	chr3:g.128,204,841_128,204,842insC *GATA2* c.599_600insG (p.Gly200Glyfs*1)	LOF ZF(-)	ID	None	None	Disseminated BCG following vaccination (12)	Bigley 2011, Dickinson 2011 (Srefs 7, 8)
Subject 2	1	*de novo*	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	ID	None	None	HPV, *M. kansasii* , bacilli and influenza H1N1 infection (27)	Bigley 2011, Dickinson 2011 (Srefs 7, 8)
Subject 3	1	Familial	p.Arg398Trp	chr3:g.128,200,113G>A *GATA2* c.1192C>T (p.Arg398Trp)	DB(-) *	ID	None	None	Pulmonary alveolar proteinosis (21)	Bigley 2011, Dickinson 2011 (Srefs 7, 8)
Subject 4	1	*de novo*	p.Ser340_Asn381del	chr3:g.128,200,788C>T *GATA2* c.1018-1G>A (p.Ser340_Asn381del)	LOF ZF(-)	ID	None	None	Recurrent erythema nodosum and HPV infection (23)	Bigley 2011, Dickinson 2011 (Srefs 7, 8)
Pedigree 1	5 Males, 8 Females	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A	None	MDS/AML, AML		Hahn 2011 (Sref 9)
Pedigree 2	4 Males, 1Female	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A	None	MDS		Hahn 2011 (Sref 9)
Pedigree 3	6 Males, 6 Females	Familial	p.Thr354Met	chr3:g.128,200,744G>A *GATA2* c.1061C>T (p.Thr354Met)	DB(-) #	M/A	None	MDS, MDS/AML, AML M2, AML M7, leukemia, macrocytic anemia	*M. avium intracellulare*	Hahn 2011 (Sref 9)
Pedigree 4	2 Males	Familial	p.355delThr	chr3:g.128,200,740-128,200,742del *GATA2* c.1063_1065delACA (p.355delThr)	DB(-) #	M/A	None	MDS		Hahn 2011 (Sref 9)

Table S2. ***Summary of genetic and clinical details of all pedigrees and individuals with GATA2 germline mutations.*** The biochemical characterization is also described. DNA binding disrupted, DB(-); DNA binding enhanced, DB(+); Protein modelling prediction, *; Zinc Finger 2 fully or partially deleted, ZF(-); Predicted loss of function, LOF; Experimentally validated, #; See proposed Structure Modelling, SM; L - lymphedema; M/A - MDS and/or AML; ID - immunodeficiency (DCML or MonoMAC); DF - dysmorphic features; NS - neurological symptoms (*e.g.* mental retardation, developmental delay); HL - hearing loss; Onset of lymphedema post surgery, §. All mutations numbered from ATG start codon of *GATA2* NM_032638.4 and NP_116027.2 (Feb. 2009 GRCh37/hg19). Lymphedema (orange); Hematopoietic malignancy (yellow); Infections (green). Without mRNA studies, the protein product cannot be predicted accurately, ¶.

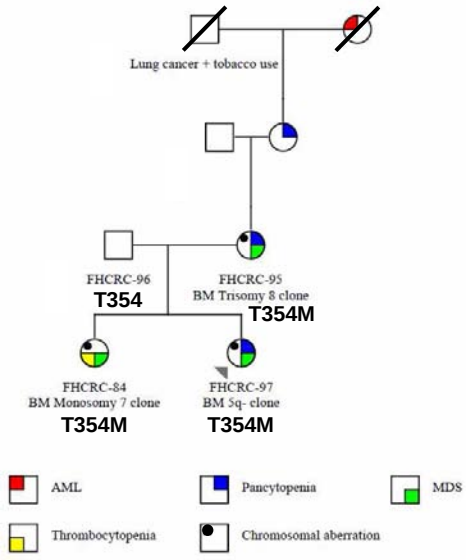
Figure S1. ***Pedigree for Patients 1, 2 and 3 carrying the GATA2 T354M mutation***

This family displays four generations of pancytopenia, MDS and AML. The *GATA2* (NM_032638.4), c.1061C>T (p.Thr354Met) (T354M) mutation was identified by whole exome sequencing and confirmed by Sanger sequencing in both directions.

Figure D2. ***Minimum overlap of 3q genomic deletions encompasses the GATA2 gene.*** Three individuals with multi-gene deletions in the 3q21 region all remove a minimum region containing the *GATA2* gene. Other genes in the region include *EEFSEC*, *DNAJB8*, *LOC90246*, *C3ORF27* (*GR6*) and *MIR1280*. Boxes with genomic coordinates (hg19) represent the maximum deleted regions.

Figure S3. ***GATA2 is present at high levels in hematopoietic cells.*** Immunostaining of E16.5 skin with antibodies to GATA2 and CD45 reveals that, in addition to localization in LEC, GATA2 is present at high levels in CD45-positive hematopoietic cells. Scale bars correspond to 60 μm.

Figure S4. ***Structural modeling of GATA2 R361L mutant.*** Human GATA2 zinc finger 2 (ZF2) shares 97% sequence identity with human and mouse GATA3. We therefore used the known murine Gata3 ZF2 structure bound to DNA to evaluate the effects of the human GATA2 R361L substitution. The four zinc coordinating cysteines are shown in magenta with the zinc ion (yellow sphere). Replacement of the positively charged arginine (red) at amino acid 361 in WT GATA2 with a hydrophobic leucine (red) (white dotted region) disrupts critical interactions (yellow dotted lines) with asparagine 371 and the GATA DNA binding site deep within the major groove (AGATAA sense strand, cyan; TTATCT antisense strand, orange; nucleotides underlined make polar contacts with R361, but not L361). Leucine 359 (blue; amino acid affected by the L359V mutation in CML blast crisis) also projects into the major groove and contacts DNA. The string of five consecutive threonines (T354-T358) is shown (yellow) and tryptophan 360 (green).



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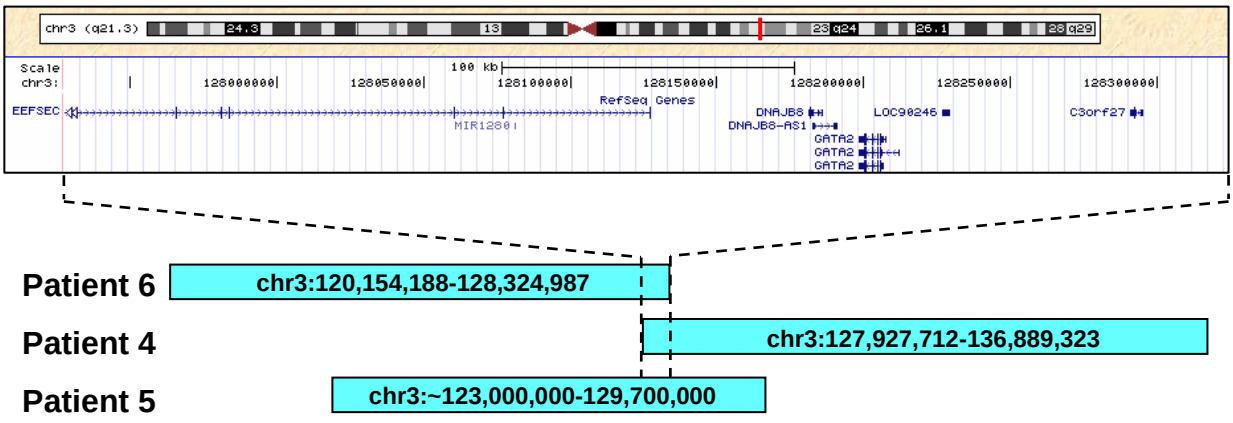

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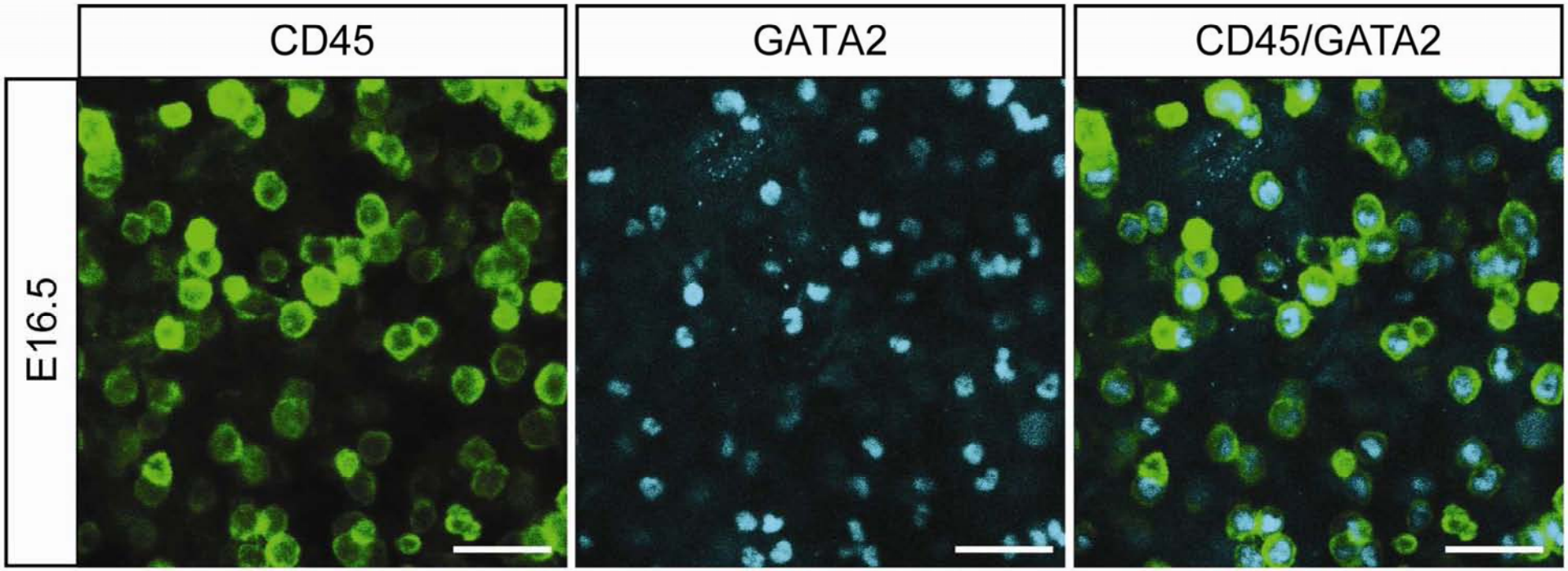
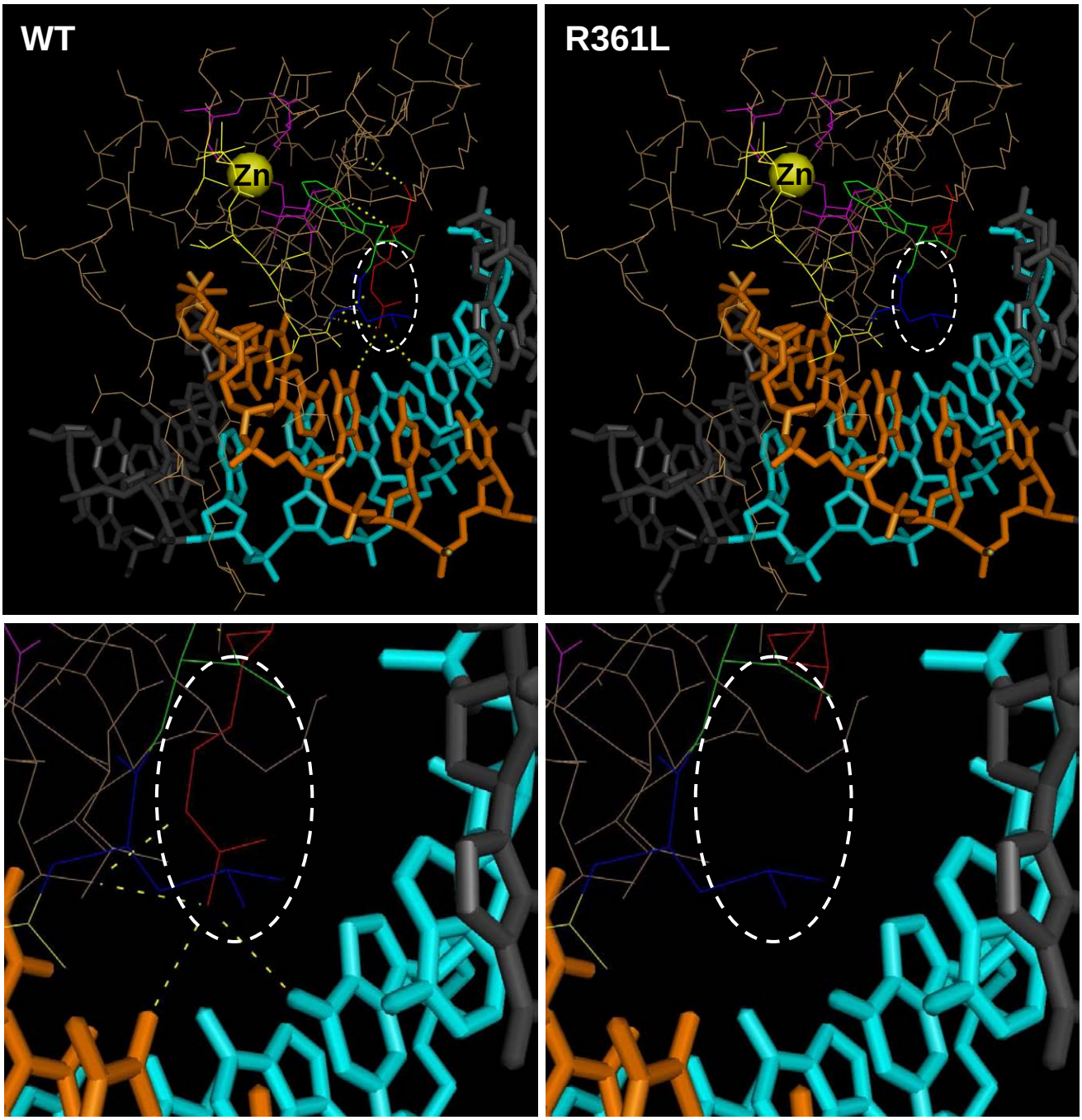
Figure S3





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