# Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients.

Jean Donadieu, Marie Lamant, Claire Fieschi, Flore Sicre de Fontbrune, Aurelie Caye, Marie Ouachee, Blandine Beaupain, Jacinta Bustamante, A Poirel Helene, Bertrand Isidor, et al.

# Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients

**Jean Donadieu,[1] Marie Lamant,[2] Claire Fieschi,[3,4] Flore Sicre de Fontbrune,[5] Aurélie Caye,[6] Marie Ouachee,[7] Blandine Beaupain,[8] Jacinta Bustamante,[9,10,11,12] Hélène A. Poirel,[13] Bertrand Isidor,[14] Eric Van Den Neste,[15] Antoine Neel,[16] Stanislas Nimubona,[17] Fabienne Toutain,[18] Vincent Barlogis,[19] Nicolas Schleinitz,[20] Thierry Leblanc,[7] Pierre Rohrlich,[21] Felipe Suarez,[22] Dana Ranta,[23] Wadih Abou Chahla,[24] Bénédicte Bruno,[24] Louis Terriou,[25] Sylvie Francois,[26] Bruno Lioure,[27] Guido Ahle,[28] Françoise Bachelerie,[29] Claude Preudhomme,[30] Eric Delabesse,[31,32] Hélène Cave,[6] Christine Bellanné-Chantelot,[33] Marlène Pasquet[2,32] and the French GATA2 study group.**

[1]Department of Paediatric Haematology and Oncology, Registre National des Neutropénies Chroniques, AP-HP Trousseau Hospital, Paris, France; [2]Department of Paediatric Haematology and Immunology, CHU Toulouse, France; [3]Department of Clinical Immunology Assistance Publique – Hôpitaux de Paris (AP-HP) Saint-Louis Hospital, France; [4]INSERM UMR1126, Centre Hayem, Université Paris Denis Diderot, Sorbonne Paris Cité, France; [5]Department of Haematology and Bone Marrow Transplantation, AP-HP Saint-Louis Hospital, Paris, France; [6]Genetic Laboratory, AP-HP Robert Debré Hospital, Paris, France; [7]Department of Haematology, AP-HP Robert Debré Hospital, Paris, France; [8]French Neutropenia Registry, AP-HP Trousseau Hospital, Paris, France; [9]Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM UMR 1163, Necker-Enfants Malades Hospital, Paris, France; [10]Centre for the Study of Primary Immunodeficiencies, Necker-Enfants Malades Hospital, AP-HP, Paris, France; [11]St Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, New York, NY, USA; [12]Paris Descartes University, Imagine Institute, Paris, France; [13]Centre for Human Genetics, Cliniques Universitaires Saint-Luc & Human Molecular Genetics (GEHU), de Duve Institute - Université Catholique de Louvain, Brussels, Belgium; [14]Department of Genetics, CHU Nantes, France; [15]Department of Haematology, St Luc Hospital, Brussels, Belgium; [16]Department of Internal Medicine, CHU Nantes, France; [17]Department of Haematology, CHU de Rennes, France; [18]Department of Paediatric Haematology and Oncology, CHU de Rennes, France; [19]Department of Paediatric Haematology, CHU de Marseille, Hopital La Timone, Université Aix-Marseille, France; [20]Internal Medicine, CHU de Marseille, Hopital La Timone, Université Aix-Marseille, France; [21]Department of Haematology, CHU de Besançon, France; [22]Department of Haematology, AP-HP Necker-Enfants Malades, INSERM UMR 1163 and CNRS ERL 8254 Institut Imagine, Sorbonne Paris Cité, Université Paris Descartes, France; [23]Department of Haematology, CHU de Nancy, France; [24]Department of Paediatric Haematology, CHU de Lille, France; [25]Department of Internal Medicine and Immunology, CHU Lille, France; [26]Department of Haematology, CHU d'Angers, France; [27]Department of Haematology, CHU de Strasbourg, France; [28]Department of Neurology, Hôpitaux Civils de Colmar, France; [29]Inflammation Chimiokines et Immunopathologie, INSERM, Faculté de Médecine, Université Paris-Sud, Université Paris-Saclay, Clamart, France; [30]Laboratory of Haematology, CHU de Lille, France; [31]Laboratory of Haematology, IUCT-Oncopole, Toulouse, France; [32]Centre of Research in Oncology, INSERM U1037, Team 16, IUCT-Oncopole, Toulouse, France and [33]Department of Genetics, AP-HP Pitié Salpêtrière Hospital, Faculté de Médecine Sorbonne Université, Paris, France

*JD and ML, and CBC and MP contributed equally to this study*

**Haematologica** 2018
Volume 103(8):1278-1287

**Correspondence:**

pasquet.m@chu-toulouse.fr

Received: October 28, 2017.
Accepted: April 27, 2018.
Pre-published: May 3, 2018.

doi:10.3324/haematol.2017.181909

*Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/103/8/1278*



## ABSTRACT

Heterozygous germline *GATA2* mutations strongly predispose to leukemia, immunodeficiency, and/or lymphoedema. We describe a series of 79 patients (53 families) diagnosed since 2011, made up of all patients in France and Belgium, with a follow up of 2249 patients/years. Median age at first clinical symptoms was 18.6 years (range, 0-61 years). Severe infectious diseases (mycobacteria, fungus, and human papilloma virus) and hematologic malignancies were the most common first manifestations. The probability of remaining symptom-free was 8% at 40 years old. Among the 53 probands, 24 had missense mutations including 4 recurrent alleles, 21 had nonsense or frameshift mutations, 4 had a whole-gene deletion, 2 had splice defects, and 2 patients had complex mutations. There were significantly more cases of leukemia in patients with missense mutations (n=14 of 34) than in

patients with nonsense or frameshift mutations (n=2 of 28). We also identify new features of the disease: acute lymphoblastic leukemia, juvenile myelomonocytic leukemia, fatal progressive multifocal leukoencephalopathy related to the JC virus, and immune/inflammatory diseases. A revised International Prognostic Scoring System (IPSS) score allowed a distinction to be made between a stable disease and hematologic transformation. Chemotherapy is of limited efficacy, and has a high toxicity with severe infectious complications. As the mortality rate is high in our cohort (up to 35% at the age of 40), hematopoietic stem cell transplantation (HSCT) remains the best choice of treatment to avoid severe infectious and/or hematologic complications. The timing of HSCT remains difficult to determine, but the earlier it is performed, the better the outcome.

## Introduction

*GATA2* gene encodes a transcription factor critical to hematopoiesis characterized by the presence of two zinc finger domains. Since 2011, heterozygous germline mutations in *GATA2* have been reported to cause a complex and heterogeneous syndrome consisting of myelodysplasia (MDS), acute myeloid leukemia (AML),[1] monocytopenia mycobacterial infections/dendritic cell,[2] monocyte, B and natural killer (NK) cell deficiency (MonoMAC[3,4]/DMLC),[5] and lymphoedema (Emberger syndrome).[6] The mutational spectrum of *GATA2* is heterogeneous, consisting of missense mutations mostly located within the highly conserved C-terminal zinc finger domains, null mutations mostly located upstream the zinc finger domains, splice site defects, mutations in the enhancer located in the intron 4,[7] and, more rarely, exonic and whole-gene deletions.

Apart from hematologic and infectious phenotypes, additional clinical presentations have been described in the last six years, such as aplastic anemia,[8] pulmonary alveolar proteinosis,[9] dermatological,[10] autoimmune or vascular features. So far, a total of 158 patients with a germline *GATA2* mutation have been reported in 4 large surveys.[11-14] Except for lymphoedema which is more frequent in patients with null or regulatory mutations, no correlation between the type or location of the *GATA2* mutation and the clinical/biological phenotype have been established in previous reports.

We now report a large multicenter survey which brings together all the patients that have been diagnosed in France and Belgium since 2011, i.e. 79 patients with a heterozygous germline *GATA2* mutation from 53 pedigrees. These 79 patients include 14 previously identified patients through the French Chronic Neutropenia Registry;[11] their follow up has been up-dated. This survey allows human GATA2 deficiency to be more accurately defined, taking advantage of a very long follow-up period (2249 patients/years). We describe the initial manifestations, their evolution, and their outcome regarding the onset of severe manifestations (leukemia, severe infections, vascular defects). This large cohort also allows new features of the disease to be described.

## Methods

### Patients

All patients with heterozygous germline *GATA2* mutations diagnosed between 2011 and 2016 in France and Belgium were enrolled in this survey, secondary to their identification through the laboratories which performed their genetic diagnosis in France and Belgium (JB, CBC, HC-AC, ED, CP). Fourteen patients with chronic neutropenia, and who were registered in the French Severe Chronic Neutropenia Registry, had been reported previously.[11] This registry has been recognized as a national registry by the French health authorities since 2008, and has contributed to several studies.[15,16] The database was approved by the French national data protection agency (CNIL, certificate n. 97.075). This registry was primary established to enrol all the patients with chronic neutropenia in France. By extension, all patients identified with a given genetic disease (e.g. *GATA2* ) occasionally associated with a chronic neutropenia can be enrolled in the registry. With regards to *GATA2* mutations, we systemically seek additional sources of enrollment, extending the borders of the initial network to internal medicine, infectious diseases and genetics, as well as from adult hematopoietic stem cell transplantation (HSCT) units.

### Genetic analysis

The patient or his/her parents gave their written informed consent to undergo genetic testing and participate in the study. Genomic DNA was extracted from a blood sample. Genetic analysis included the Sanger sequencing of exons 2 to 6, the intronic regulatory region (intron 4) of the *GATA2* gene (NM_032631.4), and the search for exonic or large genomic deletions by quantitative PCR and/or MLPA. The germline status of the identified *GATA2* mutation was confirmed by analyzing non-hematopoietic tissue (cultured skin fibroblasts, hair follicles or nails) in 30 probands. Null mutations (nonsense, frameshift, multi-exon deletion) were considered to be disease-causing. The pathogenicity of missense mutations and splice-site variants that did not affect the canonical +1 and +2 splice site bases were based on the following criteria: frequency in the general-population database [Exome Aggregation Consortium (ExAC): *http://exac.broadinstitute.org*], literature that took into account mutations that were previously reported as a *GATA2*-associated defect, functional studies supporting a damaging effect, a *de novo* occurrence, family segregation analysis, and finally predictive algorithms of pathogenicity for missense mutations [SIFT, Align GVGD, PolyPhen-2 and Combined Annotation-Dependent Depletion (CADD) score, and for splice-site defects (MaxEntScan and Human Splicing Finder)].[17]

### Clinical investigation

Demographics, immuno-hematologic parameters and infectious status were recorded. Septicemia, cellulitis, pneumonia, osteitis, and liver abscess were considered to be severe infections. Computed tomography (CT) scans, bronchioalveolar lavage and pulmonary function investigations were performed in patients with lung disease. Profuse skin or genito-anal warts were considered to be a specific event. Mycobacterial infections were considered if mycobacteria were identified in a pathological tissue (Ziehl

coloration and/or culture in 14 of 16 patients). Mycobacterial infection was suspected if the tissue sample demonstrated granuloma, and/or clinical symptoms were cured by antimycobacterial drugs (2 out of 16 patients).

Immunoglobulin levels were analyzed according to the patient's age.[18] Age at first symptom was defined by the age at the first clinical pathological manifestation among the following list: myelodysplastic syndromes (MDS) or acute leukemia (AL), any severe and potentially life-threatening infection, lymphoedema, pulmonary proteinosis, or profuse human papillomavirus (HPV) infection. A *GATA2* mutation carrier was considered asymptomatic if no clinical and/or biological symptoms were described at the last follow-up visit. Siblings or parents of probands were considered as carriers of the familial *GATA2* mutation if they presented with one of the typical manifestations of the GATA2 deficiency, even in the absence of genetic testing.

### Hematologic features

Blood counts were recorded at baseline if available, at any period following a hematologic complication, and after HSCT (if applicable). Bone marrow studies were performed in the event of blood count abnormality. Hematologic malignancies were classified according to the 2008 World Health Organization (WHO) classification.[19,20] MDS was classified according to the revised version of the International Prognostic Scoring System (IPSS)[21] and juvenile myelo-monocytic leukemia (JMML) was classified according to the 2016 WHO classification.[22]

### Statistical analysis

Stata® software (v.13) was used for all the statistical analyses. Lower and upper interquartile and median values express the distribution of quantitative variables. Differences between groups of patients were analyzed using Fisher's exact test if the event was discrete and Wilcoxon's test for quantitative variables. Survival was compared between the groups of subjects using the log-rank test, and Cox's model was used for the multivariate analysis. As we performed repeated tests, $P<0.01$ was considered significant, unless otherwise stated. For survival, the end points were death, MDS or AL; the time-period started at birth until an event or the day of last news. We also analyzed survival after onset of a clonal event. The time period started from the first clonal event (MDS or AL) until death or the day of last news. The Kaplan-Meier method was used to estimate survival rates. The cut-off date was 30th September 2016.

## Results

### Early onset of severe infections and/or hematologic diseases in GATA2 deficiency

Forty males and 39 females from 53 families with a heterozygous germline *GATA2* mutation are herein reported (Table 1 and *Online Supplementary Table S1*), including 14 previously described patients,[11] whose clinical and biological data have been up-dated. The patients were enrolled in France (n=72) and Belgium (n=7). Median age at the last follow up was 24.5 years old (range, 3.9-73). The probability of remaining symptom-free was 38% at the age of 20 (95%CI: 27-48.7%) and 8% at the age of 40 (95%CI: 3.3-15%) (Figure 1A). All patients except 5 were symptomatic at the time of the last follow up. These 5 individuals were first-degree relatives of symptomatic patients with a *GATA2* mutation (*Online Supplementary Table S2*). Median age at onset of the first clinical symptom was 18.6 years (range, 0-61) (Figure 1A and *Online Supplementary Table S1*). Initial manifestations were a hematologic malignancy in 19 patients (26%), a severe bacterial infection in 17 (23%), profuse warts or HPV in 15 (20%), lymphoedema in 7 (9.4%), or a mycobacterial infectious disease in 6 (8.1%). Blood counts of patients with opportunistic infections (HPV, mycobacteria, mycosis, the JC virus) were systematically abnormal (monocytopenia, neutropenia, pancytopenia, severe anemia).

### Additional clinical features in GATA2 deficiency

Outside hematologic and infectious clinical presentations, erythema nodosum/panniculitis (4 patients), mental retardation (1 patient), transient ischemic cerebral palsy (1 patient), and progressive multifocal leukoencephalopathy linked to the JC virus (PML, 1 patient) were the initial symptoms in 7 patients.

Over the course of the disease, 9 patients had systemic inflammatory manifestations with panniculitis, vasculitis, Sweet's syndrome, lupus-like disease or granulomatous disease mimicking sarcoidosis. Of note, auto-immune markers were present in 12 patients, which may be an underestimation because they were not sought for in all patients (Table 1).

Chronic lymphoedema was noted in 12 patients (15%). Vascular and/or thrombotic complications were observed in 7 patients: 2 patients presented with transient cerebral palsy, one patient presented with splenic-vein thrombosis after a splenectomy and mycobacteriosis, one patient presented with 3 deep-vein thromboses in a context of AL,

**Table 1. Clinical and biological presentation of *GATA2* deficient patients.**

| Diagnostic features | Clinical and biological aspects | Incidence in our survey |
|---|---|---|
| Hematologic features | MDS | 70% (55/79) |
| | AML | 19% (15/79) |
| | ALL | 1.3% (1/79) |
| | Aplastic anemia | 2.5% (2/79) |
| | Juvenile myelomonocytic leukemia | 1.3% (1/79) |
| Recurrent infections (viral, mycobacterial, fungal) | Monocytopenia | 49% (24/49) |
| | B lymphopenia | 100% (38/38) |
| | NK lymphopenia | 7.8% (3/38) |
| Warts | HPV-related (genital and cutaneous) Oncogenesis | 40% (32/79) |
| | | 3.8% (3/79) |
| Lymphoedema | | 15% (12/79) |
| Pulmonary features | Pulmonary alveolar proteinosis | 3.8% (3/79) |
| | Recurrent bacterial infections | 56% (44/79) |
| Vascular features | Thrombosis, myocardial infarction | 9% (7/79) |
| Deafness | | 1.3% (1/79) |
| Autoimmune features | Panniculitis, erythema nodosum, vasculatis, lupus-like and sarcoidosis-like syndrome, Sweet's syndrome | 11% (9/79) |
| Other features | Urinary-system malformation | 5% (4/79) |
| | Premature labor, miscarriage | 6.3% (5/79) |
| | Hypothyroidism | 1.3% (1/79) |

MDS: myelodysplastic syndromes; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; NK: natural killer cell; HPV: human papillomavirus.

one patient presented with deep-vein thrombosis and pulmonary embolism while receiving treatment for breast cancer and MDS, one patient presented with myocardial infarction at the age of 40, and one patient died from aortic dissection at the age of 33 years (Table 1).

Only 3 patients had pulmonary alveolar proteinosis and one patient in the cohort was deaf. Four patients had urogenital abnormalities. Three patients had been born prematurely, 2 women suffered from miscarriages, and one patient had hypothyroidism.

### At diagnosis, the majority of GATA2 deficient patients had abnormal blood parameters

Sixty of the 74 symptomatic patients were free of hematologic malignancy at diagnosis. A blood count was available for 49 patients before hematologic evolution. The

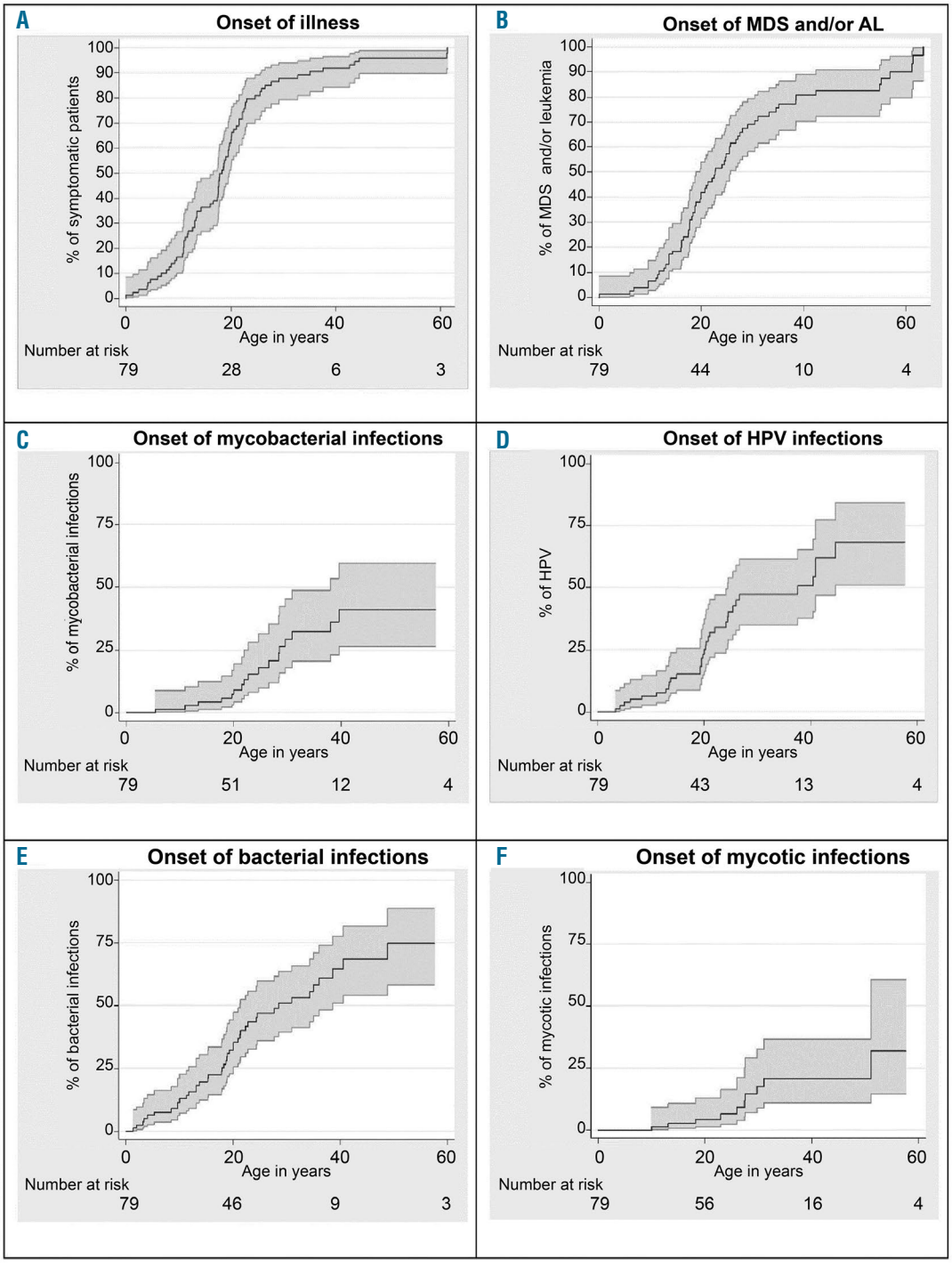


**Figure 1. Onset of disease, hematologic and infectious complications.** (A) Kaplan Meier curves are shown for the onset of disease in 74 symptomatic patients with a *GATA2* mutation. (B) Occurrence of myelodysplastic leukemia (MDS) / acute leukemia (AL).(C-F) Rate in mycobacterial, human papillomavirus (HPV), bacterial and mycotic infections. Confidence intervals of 95% are shaded gray.

blood count was abnormal in 36 patients (73%): 24 patients (49%) had monocytopenia lower than 0.1 G/L, 19 patients (39%) had neutropenia lower than 1.5 G/L, 9 patients (18%) had platelet levels lower than $100x10^9$/L, 7 patients (14%) had macrocytosis, and 5 patients (10.2%) had anemia lower than 9 g/dL (*Online Supplementary Figure S1*). Consequently, 36 out of these 49 evaluable patients (73%) had an abnormal blood count.

Immunological data were available in 38 patients: T-cell counts were slightly decreased (median 0.97 G/L T CD3 (range, 0.1-7.5), 0.37 G/L T CD4 (range, 0.05-5.6), and 0.49 G/L T CD8 (range, 0.02-2.3), NK cells ($CD16^+/CD56^+$) were preserved (median 0.12 G/L, range: 0-0.34); B-cell levels were consistently low (median 0.02 G/L, range, 0-1.51) although immunoglobulin levels were within normal ranges (median IgG=9.3 g/L, range, 4-40; IgA=0.9 g/L, range, 0.33-3.4; IgM=1 g/L, range, 0.05-2.40). Overall, GATA2 defects are mainly associated with a monocytopenia and a B-cell lymphopenia.

## More than 80% of patients presented with a hematologic malignancy at the age of 40 years

Among the 74 symptomatic patients, 64 developed a hematologic malignancy (MDS and/or AL). The risk of developing MDS/AL rapidly increased from 6% at the age of 10 years to 39% at the age of 20, and 81% at the age of 40 (Figure 1B). Among the 64 patients, the initial diagnosis was MDS in 55 patients (69%), AL in 7 patients (9%), and chronic leukemia in 2 patients (3%). Among the 55 patients with an MDS, a progression to AL was observed in 9 patients (16%) (Figure 2A). The AL were mainly myeloid (AML), but we observed a case of T-cell acute lymphoblastic leukemia with a monosomy 7. In addition to these hematologic complications, a juvenile myelomonocytic leukemia (JMML) case occurred in a neonate. This patient received neither chemotherapy nor allograft. This patient's blood count is normal four years after diagnosis without any treatment.

Karyotypes were abnormal in 43 of 66 patients (65%), with a complete or a partial loss of chromosome 7 in 27 cases (35%), trisomy-8 in 16 cases (18%), 4 patients combining the two (Figure 2B).

In order to better define the prognosis of MDS, the IPSS-R[21] was calculated for 47 of 55 patients upon diagnosis of MDS. The prognosis was mainly intermediate (1.5-4.5) in 24 patients (51%), high (>4.5) in 13 patients (27%), and low (<1.5) in 10 patients (21%). There was no significant difference in the age of the patients between these 3 groups.

## Low frequency of solid neoplasia

Solid tumors were identified in 6 patients only, mainly secondary to HPV (3 cases). In addition, one woman developed breast cancer, one patient developed a metastatic adenocarcinoma, and one patient developed an epidermoid carcinoma.

## Severe infectious diseases explain the high mortality

Severe bacterial infections were the most frequent feature, occurring at some time over the patients' lives in 44 cases (56%). The 20-year cumulative rate of bacterial infection was 33%, rising to 64% at the age of 40 years (Figure 1E). Lung infections were the most frequent (two-thirds of all cases) and, although they evolved

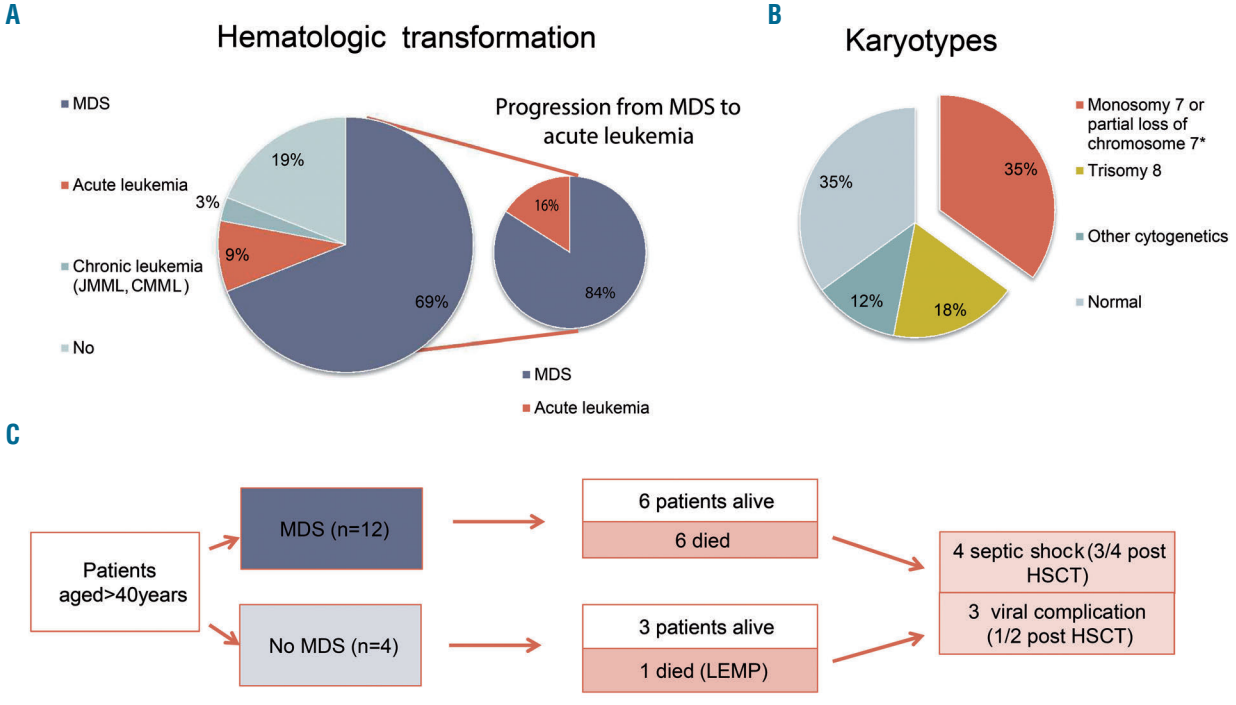


**Figure 2. Hematologic features of the 79 patients.** (A) Hematologic malignancies among the 79 patients. Progression to acute myeloid leukemia (AML) from myelodysplastic syndromes (MDS) is indicated. (B) Karyotypes availables for 66 out of 79 *GATA2*-deficient patients. *4 patients with monosomy 7 also had trisomy 8. (C) Hematologic complications and the outcome of patients older than the age of 40. JMML: juvenile myelomonocytic leukemia; CMML: chronic myelomonocytic leukemia; LEMP: leukoencephalomyelopathy; HSCT: hematopoietic stem cell transplantation.

favorably with antibiotics, recurrences were frequent. Nine patients had bacterial soft-tissue infections, and 5 had ENT infections. Twelve patients had a non-tuberculous mycobacterial infection (Figures 1C and 3) (*Mycobacterium avium, kansasii, chelonae, genavense*), and 4 patients developed tuberculosis. These mycobacterial infections were concomitant with MDS in 7 cases. The risk of acquiring a mycobacterial infection increased with age: 9% at the age of 20 years to 42% at the age of 40 years.

Severe viral infections led to death in 4 patients: H1N1 influenza five years after AML treatment (Figure 3), Epstein-Barr virus (EBV) lymphoproliferative disease after HSCT, HPV-related metastatic carcinoma and a progressive multifocal leukoencephalopathy caused by the JC virus which was the first manifestation of the disease. Cutaneous or genital recurrent HPV-induced warts were often the first reported symptom (32 cases, 40%), with 20-year and 40-year rates of 25% and 50%, respectively (Figures 1 and 3). A high resistance to local treatment and frequent recurrences were common. Two patients developed a neoplasia.

Eighteen fungal infections were observed in 16 patients (11 cases of aspergillosis, 5 of candidosis, and 2 of mucormycosis). Eight of these 18 infections were diagnosed during chemotherapy (n=5) or HSCT (n=3) (Figure 1F).

Several infectious complications appeared post HSCT (3 fungal, 1 viral and 2 bacterial infections related to HSV, 2 patients with EBV prior to the HSCT had recurrence of this virus after HSCT, which evolved to lymphoproliferative disease in 1 patient) (*Online Supplementary Table S1*).

The course of infection was complicated by hemophagocytic syndrome in 6 patients (2 mycobacterial, 1 fungal and 3 viral infections).

## A poor survival rate was observed in GATA2-deficient patients despite aggressive treatments

In our cohort, 27 patients (34%) died at a median age of

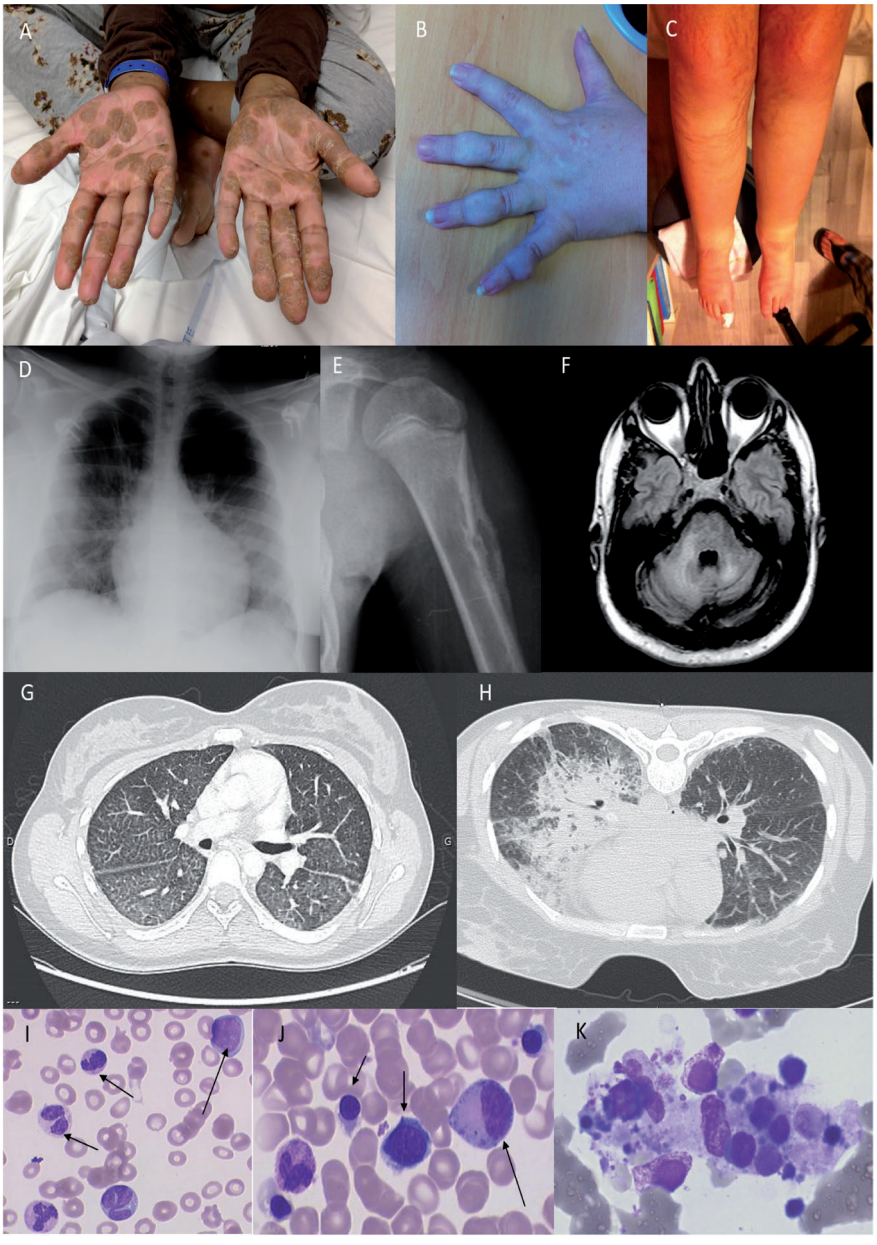


**Figure 3. Clinical, radiographic, and cytological features of *GATA2* syndrome.** (A) Cutaneous warts on the hands of a woman with myelodysplastic syndromes (MDS). (B) Hand rheumatism (C) Bilateral lymphoedema post-hematopoietic stem cell transplantation. (D) Acute respiratory distress syndrome in a H1N1 infection. (E) Osteomyelitis at presentation. (F) Progressive multifocal leukoencephalopathy in a 43-year old man. (G) Pulmonary alveolar proteinosis in a woman with MDS and warts. (H) Disseminated mycobacteriosis. (I and J) Bone marrow smears of pedigree 46. (I) Dysgranulopoiesis and blasts in a woman with MDS evolving to acute myeloid leukemia.[2] (J) Dyserythropoiesis and dysgranulopoiesis in her son with MDS. (K) Macrophage activation secondary to flu infection.

29 years (range, 10.2-72.6). Survival analyses demonstrated a poor outcome: mortality was 6% at the age of 20, 42% at the age of 40, and 69% at the age of 60 (Figure 4A). Probability of survival after a clonal event (MDS and/or AL) was 60% by the age of 40 (Figure 4B). The 5-year survival rate in patients with MDS regarding the 3 IPSS-R groups was: 30% in the high-risk group, 80% in the intermediate-risk group, and 100% in the low-risk group ($P$<0.001) (Figure 4D). Of note, severe bacterial and/or viral complications were the main causes of death in patients over the age of 40 (Figure 2C).

Myelodysplastic syndromes and AL were the main causes of death in 15 patients: 8 cases after chemotherapy, and 7 after HSCT. Ten patients had lethal infections: disseminated mycobacterial infections in 3, bacterial infections in 3, and severe viral infections in 4 (JC virus encephalitis, oncogenic HPV, H1N1 flu, and EBV lymphoproliferative disease post HSCT). One patient died from an aortic dissection and another from metastatic carcinoma.

Twenty-eight patients underwent HSCT for MDS or AL and/or immune deficiency. The overall survival rate of these patients was 73% after one year and 62% after five years, which then plateaued. Nine of the 28 patients died from severe infections or graft-*versus*-host disease. Survival after HSCT was dependent on the age at transplantation (Figure 4): the earlier the HSCT was performed, the better the outcome, even if the difference was not statistically significant.

In cases of AL (n=18), an aggressive chemotherapy induction regimen was proposed for 16 patients, with primary failure in 12 and severe infectious toxicity in 9 cases (5 cases of fungal infection). A demethylating agent was given to 3 patients and has allowed long-term disease management for MDS (n=1) and AML (n=2).

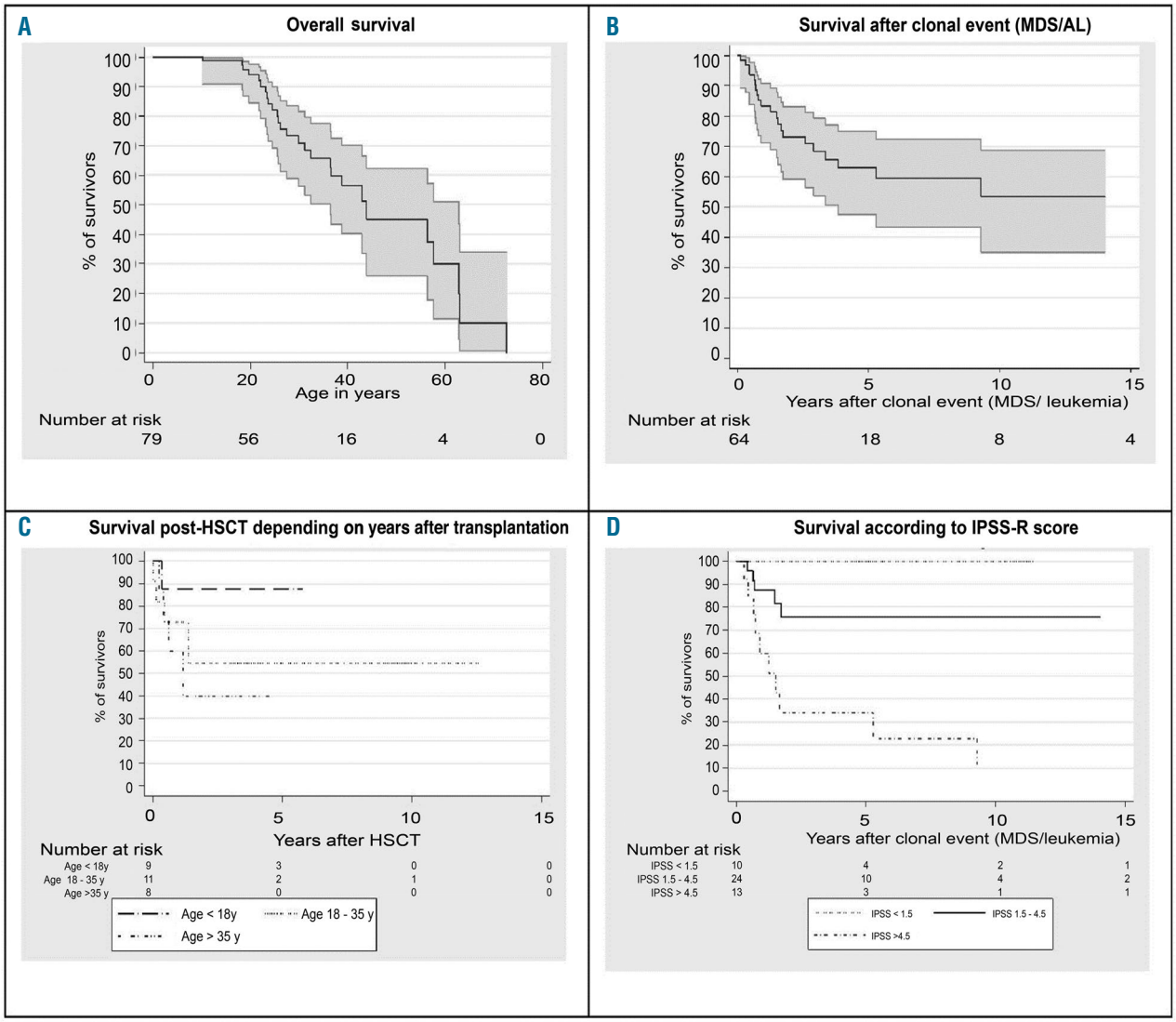


**Figure 4. Survival in 79 patients.** (A) Kaplan-Meier curves showing overall survival of the whole cohort. (B) Survival after a clonal event. (C) Overall survival was studied after hematopoietic stem cell transplantation (HSCT) depending on the age (years) at transplantation ($P$>0.05). (D) Overall survival of patients with myelodysplastic syndromes (MDS) and/or acute leukemia (AL) was plotted according to the revised International Prognostic Scoring System (IPSS-R) score at the time of the diagnosis of the malignancy. Survival significantly depends on the IPSS score ($P$<0.001). Confidence intervals of 95% are shaded gray. y: years.

### Genotype/phenotype correlation: leukemia was more frequently observed in patients with missense mutations

Among the 53 probands, 45 different mutated alleles were identified (*Online Supplementary Table S1*). Mutations were mainly located in exons 4 and 5 (Figure 5). Four patients (8% of the cohort) had a complete heterozygous *GATA2* locus deletion. Five mutations were recurrent in unrelated families (R362X, R361H, A372T, R396W, R398Q). Some residues tended to be mutated: T354 (P or R), R361 (G or H), R362 (P or X), R396 (W or Q), R398 (W or Q) (Figure 5). We identified 19 different missense mutations in 24 probands and 14 relatives (46%), 7 nonsense mutations in 10 probands and 4 relatives (17%), and 11 small deletions or insertions leading to predicted stop codons (21%). There were 2 splice defects (4%), one in frame duplication (2%) and one intronic variant (2%) located in the regulatory element of intron 4, (Figure 5 and *Online Supplementary Table S1*). The germline status of the GATA2 mutations was confirmed in 30 probands. The other 23 mutations were highly suspected to be germline as the variant allele frequency was close to 50%. Parental segregation was analyzed in 27 pedigrees. In 6 probands, the *GATA2* mutation occurred *de novo* (P1, P9, P33, P35, P47 and P52).

There was no significant difference in median age at diagnosis between probands and relatives. If we consider 2 groups of mutations based on the type of mutation (missense *vs.* nonsense/frameshift), no genotype/phenotype correlation could be highlighted regarding infection, warts, MDS, neoplasia or inflammatory complications. By contrast, there was a significant risk of developing leukemia in the group of patients with the missense mutations (14 of 38) *versus* the group with nonsense or frameshift mutations (2 of 28; $P$=0.007, Fisher's exact test) (Table 2).

## Discussion

This large cohort with germline *GATA2* mutation has the longest follow up (2249 patients/year) of any study. The homogeneous and exhaustive available clinical and biological data allow key clinical points regarding the disease to be described: the majority of patients (>90%) will present with a life-threatening hematologic and/or infectious manifestation by the age of 40. Within the first decade, disease presentation is limited to common bacterial infections or lymphoedema. During the second decade, patients may present with infections and/or inflammatory disease and/or hematologic transformation. Monocytopenia was frequent even without any other detectable hematologic disease.

Our study also confirmed that hematologic complications are the major issue of the GATA2 deficiency: the probability of developing MDS and/or AML rapidly increased from 39% at the age of 20 to 80% at the age of

**Table 2.** Genotype/phenotype associations. The missense mutation group was associated with a significant risk of leukemia (*$P$=0.007, Fisher's exact test). MDS: myelodysplasia.

| Patient pedigree | Leukemia | MDS | Warts | Fungus | Mycobacteria |
|---|---|---|---|---|---|
| Missense mutations (n=38) | 14 | 27 | 11 | 8 | 6 |
| Frameshift + nonsense mutations (n=28) | 2 | 20 | 15 | 3 | 8 |
| $P$ | 0.007* | 0.793 | 0.081 | 0.331 | 0.237 |

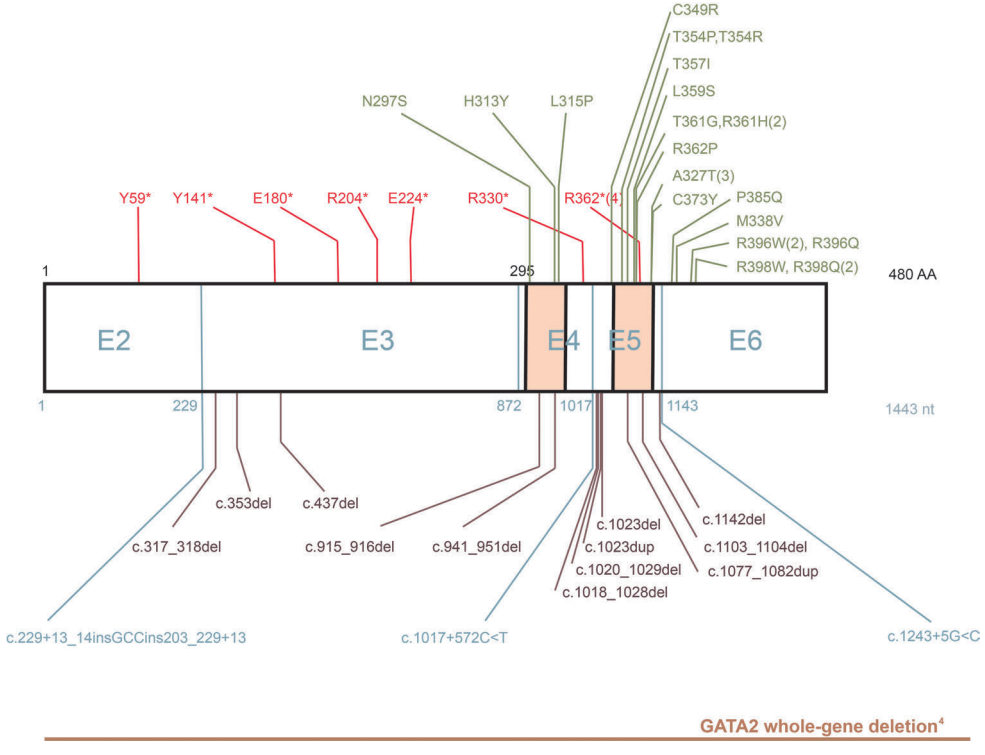


**Figure 5. Schematic organization of the *GATA2* locus and protein.** The protein is composed of 480 amino acids (top) encoded by 1443 nucleotides (bottom). The 5 coding exons (E2 to E6) are separated by blue lines. Forty-one mutations are shown: whole locus deletion including the del3q21 are in orange, nonsense mutation in the top of the schematic protein is in red, missense mutations are in green, small deletion in the bottom of the scheme is in brown, intronic mutation and splice defects are in blue, at the bottom, and mutation of the enhancer is in dark blue. Recurrence of mutations is specified in brackets.

40. Eighteen patients developed acute or chronic leukemia. In addition, we identified a second patient with acute lymphoblastic leukemia (ALL).[23] The GATA2 transcription factor is crucial for hematopoietic stem cell self-renewal and differentiation,[24,25] but also for B- and T-cell development *in vitro*, as shown in a recent murine low-level GATA2 overexpression model.[26] Only 4 of 18 patients survived (2 after HSCT, one after azacitidine treatment, one JMML). Most of the other 14 died from infections and/or progressive hematologic disease. Long-term survival of our cohort is poor, with a high rate of mortality (probability of 42% at the age of 40, 69% at the age of 60). Classic chemotherapy strategies were revealed to be toxic and poorly efficient, and HSCT is hampered by the very high rates of toxicity in these patients.

Early deaths were caused by the association of hematologic malignancies with severe infections. We propose to identify the patients at risk of evolution towards leukemia using the IPSS-R score.[21] Moreover, secondary somatic mutations occur, which leads to leukemic transformation in patients with a *GATA2* mutation. *ASXL1* mutations were implicated in the first reports,[27,28] then mutations in the RAS pathway and in the AML/MDS mutated genes.[29] Some patients have a long history of low-risk MDS before it evolves into an aggressive disease, thus underlying the importance of identifying the markers that precede this hematologic evolution to help clinicians. Importantly, our study showed that the earlier HSCT is performed, the better the outcome. The question of timing of pre-emptive transplantation is still a subject of debate, but the improved overall survival of patients with refractory cytopenias suggests that early HSCT is a reasonable approach. Identification of additional somatic mutation in patients with MDS may prompt clinicians to perform HSCT.

Our series confirmed the heterogeneity of the *GATA2* mutational spectrum, with 45 different alleles including 26 new mutations. The previously published series have not reported correlations between genotype and phenotype, with the exception of null mutants which seems to be associated with an increased risk of lymphoedema in the US cohort.[15] In our cohort, patients with missense mutations had a higher risk of developing leukemia than patients with frameshift or nonsense mutations. These data may suggest that the translated mutated GATA2 protein resulting from missense mutations is dominant negative and/or promotes leukemogenesis in contrast to frameshift or nonsense mutations, which may lead to haploinsufficiency. Recently, Chong *et al.* reported that the most prevalent GATA2 missense mutations (gT354M, gR396Q and gR398W) exhibit differences in the age of leukemia onset, supporting the concept of different functional consequences of GATA2 mutants.[30] Our observation is reported for the first time and may also help clinicians to choose the best therapeutic option, especially an aggressive treatment for the disease. Further functional studies are needed to demonstrate this hypothesis.

Severe and recurrent bacterial infections are frequent at diagnosis, and persist throughout the patient's life. A mild defect of immunoglobulin production or a weak vaccinal response had also been reported in patients with a GATA2 deficiency.[31] There is a lower incidence of mycobacterial diseases in our cohort (40% of the patients at the age of 40) than previously reported,[13,14] occurring after the age of 20 in the majority of patients. All patients with mycobacterial disease have abnormal blood counts, monocytopenia (10 of 16), MDS (9 of 15) or both (7 of 15). The relatively low frequency of mycobacterial infection may be explained by the severity of disseminated infection leading to death or drastic treatment (such as HSCT) to avoid recurrence. Some patients experienced successive diseases with different species of environmental mycobacteria, suggesting that immunological memory is not efficient in patients with *GATA2* mutations. Fungal infections occurred in 18 patients. Aspergillosis was always associated with neutropenia, as a consequence of GATA2 deficiency or secondary to the chemotherapy.

Multiple cutaneous and genital warts at presentation are frequent (32 patients). Recurrent and life-threatening oncogenic HPV lesions led us to recommend early HPV vaccination, as proposed in WILD syndrome,[32,33] which is maybe a clinical variant of GATA2 deficiency.[34] Interestingly, one patient developed new HPV lesions after HSCT, raising the question of HPV genome persistence in epithelial cells, or a specific role for GATA2 in keratinocytes in the host control of HPV. It suggests that early HPV vaccination should be proposed in mutated patients. Susceptibility to severe viral infections led to 4 deaths in our cohort. One patient died from PML caused by the JC virus as the first manifestation of GATA2 deficiency; NK cell deficiency,[35] monocytopenia and dendritic cell deficiency[5] probably contribute to this immunodeficiency.

New clinical presentations were identified in our survey. Auto-immune or chronic inflammatory disorders, such as lupus, sarcoidosis-like disease, Sweet's syndrome, panniculitis are recurrent. Lupus-like symptoms and autoimmune hepatitis have also been described in *GATA2* deficiency.[14,36] Given the occurrence of mycobacterial disease, infection should be investigated in patients with proven granuloma.

Beyond the marked clinical heterogeneity of GATA2 deficiency, we also described 5 asymptomatic cases, including that of a 60-year old patient, raising the possibility that clinical penetrance is not complete. To evaluate clinical penetrance, genotypes of all first degree relatives of patients must be available. Moreover, these observations should lead to systematically testing a potential relative considered for donation when an HSCT with a sibling donor is feasible.

This multicenter study was a unique opportunity to provide an extended and detailed clinical picture of GATA2 deficiency, which is a severe disorder that combines immunodeficiency, hematologic malignancy, pulmonary, dermatological and vascular diseases. It highlighted the fact that patients with *GATA2* missense mutations have a high risk of developing leukemia and that this may be prevented by early HSCT with the help of new markers (identification of additional somatic mutations).

***Acknowledgments***

*The Authors thank the patients and families for their participation in this study. The French registry is supported by grants from Amgen SAS, Chugai SA, Novartis, and by a grant from the Inserm. This project is supported by grants from Associations Laurette Fugain, 111 les Arts, Société Française des Cancers de l'Enfant, Enfanfare, Association Sportive de Saint Quentin Fallavier, and Barth France. The Authors thank the association IRIS for its support.*

## Reference


1. Hahn CN, Chong C-E, Carmichael CL, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet. 2011;43(10):1012-1017.
2. Vinh DC, Patel SY, Uzel G, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood. 2010;115(8):1519-1529.
3. Calvo KR, Vinh DC, Maric I, et al. Myelodysplasia in autosomal dominant and sporadic monocytopenia immunodeficiency syndrome: diagnostic features and clinical implications. Haematologica. 2011;96(8):1221-1225.
4. Hsu AP, Sampaio EP, Khan J, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood. 2011;118(10):2653-2655.
5. Bigley V, Haniffa M, Doulatov S, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. J Exp Med. 2011;208(2):227-234.
6. Ostergaard P, Simpson MA, Connell FC, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet. 2011;43(10):929-931.
7. Hsu AP, Johnson KD, Falcone EL, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. Blood. 2013;121(19):3830-3837, S1-7.
8. Ganapathi KA, Townsley DM, Hsu AP, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. Blood. 2015;125(1):56-70.
9. Griese M, Zarbock R, Costabel U, et al. GATA2 deficiency in children and adults with severe pulmonary alveolar proteinosis and hematologic disorders. BMC Pulm Med. 2015;1587.
10. Polat A, Dinulescu M, Fraitag S, et al. Skin manifestations among GATA2-deficient patients. Br J Dermatol. 2018;178(3):781-785.
11. Pasquet M, Bellanné-Chantelot C, Tavitian S, et al. High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. Blood. 2013;121(5):822-829.
12. Wlodarski MW, Hirabayashi S, Pastor V, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127(11):1387-1397; quiz 1518.
13. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809-821.
14. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol. 2015;169(2):173-187.
15. Donadieu J, Beaupain B, Mahlaoui N, Bellanné-Chantelot C. Epidemiology of congenital neutropenia. Hematol Oncol Clin North Am. 2013;27(1):1-17, vii.
16. Donadieu J, Leblanc T, Bader Meunier B, et al. Analysis of risk factors for myelodysplasias, leukemias and death from infection among patients with congenital neutropenia. Experience of the French Severe Chronic Neutropenia Study Group. Haematologica. 2005;90(1):45-53.
17. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med Off J Am Coll Med Genet. 2015;17(5):405-424.
18. Plebani A, Ugazio AG, Avanzini MA, et al. Serum IgG subclass concentrations in healthy subjects at different age: age normal percentile charts. Eur J Pediatr. 1989;149(3):164-167.
19. Niemeyer CM, Baumann I. Classification of childhood aplastic anemia and myelodysplastic syndrome. Hematol Am Soc Hematol Educ Program. 2011;2011:84-89.
20. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-951.
21. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454-2465.
22. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
23. Koegel AK, Hofmann I, Moffitt K, Degar B, Duncan C, Tubman VN. Acute lymphoblastic leukemia in a patient with MonoMAC syndrome/GATA2 haploinsufficiency. Pediatr Blood Cancer. 2016;63(10):1844-1847.
24. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature. 1994;371(6494):221-226.
25. Rodrigues NP, Tipping AJ, Wang Z, Enver T. GATA-2 mediated regulation of normal hematopoietic stem/progenitor cell function, myelodysplasia and myeloid leukemia. Int J Biochem Cell Biol. 2012;44(3):457-460.
26. Nandakumar SK, Johnson K, Throm SL, Pestina TI, Neale G, Persons DA. Low-level GATA2 overexpression promotes myeloid progenitor self-renewal and blocks lymphoid differentiation in mice. Exp Hematol. 2015;43(7):565-577.e1-10.
27. Bödör C, Renneville A, Smith M, et al. Germ-line GATA2 p.THR354MET mutation in familial myelodysplastic syndrome with acquired monosomy 7 and ASXL1 mutation demonstrating rapid onset and poor survival. Haematologica. 2012;97(6):890-894.
28. West RR, Hsu AP, Holland SM, Cuellar-Rodriguez J, Hickstein DD. Acquired ASXL1 mutations are common in patients with inherited GATA2 mutations and correlate with myeloid transformation. Haematologica. 2014;99(2):276-281.
29. Wang X, Muramatsu H, Okuno Y, et al. GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies. Haematologica. 2015;100(10):e398-401.
30. Chong C-E, Venugopal P, Stokes PH, et al. Differential effects on gene transcription and hematopoietic differentiation correlate with GATA2 mutant disease phenotypes. Leukemia. 2018;32(1):194-202.
31. Chou J, Lutskiy M, Tsitsikov E, Notarangelo LD, Geha RS, Dioun A. Presence of hypogammaglobulinemia and abnormal antibody responses in GATA2 deficiency. J Allergy Clin Immunol. 2014;134(1):223-226.
32. Kreuter A, Hochdorfer B, Brockmeyer NH, et al. A human papillomavirus-associated disease with disseminated warts, depressed cell-mediated immunity, primary lymphedema, and anogenital dysplasia: WILD syndrome. Arch Dermatol. 2008;144(3):366-372.
33. Ostrow RS, Manias D, Mitchell AJ, Stawowy L, Faras AJ. Epidermodysplasia verruciformis. A case associated with primary lymphatic dysplasia, depressed cell-mediated immunity, and Bowen's disease containing human papillomavirus 16 DNA. Arch Dermatol. 1987;123(11):1511-1516.
34. Dorn JM, Patnaik MS, Van Hee M, et al. WILD syndrome is GATA2 deficiency: A novel deletion in the GATA2 gene. J Allergy Clin Immunol Pract. 2017;5(4):1149-1152.e1.
35. Mace EM, Hsu AP, Monaco-Shawver L, et al. Mutations in GATA2 cause human NK cell deficiency with specific loss of the CD56(bright) subset. Blood. 2013;121(14):2669-2677.
36. Webb G, Chen Y-Y, Li K-K, et al. Single-gene association between GATA-2 and autoimmune hepatitis: A novel genetic insight highlighting immunologic pathways to disease. J Hepatol. 2016;64(5):1190-1193.