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ORIGINAL ARTICLE

GATA2 gene analysis in several forms of hematological malignancies including familial aggregations

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Abstract The genetic predisposition to familial hematological malignancies has been previously reported highlighting inherited gene mutations. Several genes have been reported but genetic basis remains not well defined. In this study, we extended our investigation to a potential candidate *GATA2* gene which was analyzed by direct sequencing in 119 cases including familial aggregations with a variety of hematological malignancies and sporadic acute leukemia belonging to Tunisian and French populations. We reported a deleterious *p.Arg396Gln GATA2* mutation in one patient diagnosed with both sporadic acute myeloid leukemia (AML) and breast cancer. We also reported several *GATA2* variations in familial cases. The absence of deleterious mutations in this large cohort of familial aggregations of hematological malignancies may strengthen the hypothesis that *GATA2* mutations are an important predisposing factor, although as a secondary genetic event, required for the development of overt malignant disease.

Keywords *GATA2* gene · Gene mutations · Familial hematological malignancies · Sporadic acute leukemia

Introduction

Several studies have reported that genetic predisposition to familial hematological malignancies (FHM) may lead to an increased risk of this pathology. Owing to the molecular progress in the last decades, many genes have been identified in association with familial aggregations of hematological malignancies. Due to the heterogeneity of the identified genes, the predisposition to FHM remains difficult to identify. In fact, the incomplete penetrance of the involved genes leads to overlapping and variable phenotypes depending on the studied familial aggregation. The lack of large families with multiple cases of hematological malignancies and unavailability of complete familial history enhance the complexity of the inherited factor identification. The important advances in molecular tests have led to the discovery of several genes

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recurrently mutated in familial hematological malignancies such as *GATA2* [1–4]. *GATA2* gene, located on chromosome 3q21.3, encodes a zing-finger transcription factor which is crucial for hematopoiesis, especially in regulating the development and proliferation of early pluripotent hematopoietic precursors [5].

Previous studies have reported several mutations in *GATA2* gene including dominant-negative mutations and loss-of-function mutations that lead to haploinsufficiency. These mutations such as *p.Thr354Met* have been found related to several hematological malignancies specifically in familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) aggregations [6]. Moreover, epidemiological analysis showed that individuals carrying germline *GATA2* mutation have an increased risk (~70%) of developing MDS/AML or chronic myeloid leukemia (CML) syndromes [1, 6–8].

Besides germline mutations in *GATA2* gene, somatic mutations have been also described in *CEBPA* gene in several hematological malignancies including basically myeloid lineage such as AML and CML syndromes [9, 10].

Since numerous mutations have been found in *GATA2* gene in association with mostly the familial aggregations of MDS/AML, this gene is highly suspected to be involved in the predisposition to familial hematological malignancies. In this study, we evaluate the mutational status of *GATA2* gene in familial aggregations of hematological malignancies and sporadic acute leukemia belonging to Tunisian and French populations.

Materials and methods

Patients

A total of 119 patients diagnosed with hematological malignancies, including 98 familial cases and 21 sporadic cases, were recruited via a French national cooperative network focusing on FMH and through the GenHe-mINSERM/DGRS French-Tunisian project.

The hematological malignancies cohort of patients includes chronic or acute, lymphoid or myeloid leukemia, Hodgkin's or non Hogdkin's lymphomas, and myeloproliferative or myelodysplastic syndromes.

The study cohort consists of 80 patients belonging to 71 familial forms of hematological malignancies (at least two cases of hematological malignancies with or without solid tumors in first, second, or third degree relatives); 17 patients from 17 families with aggregation of tumors including one case of hematological malignancy in first, second, or third degree relatives and 1 patient who had a multiple primitive tumor with hematological malignancy but without familial history. Thirteen sporadic acute lymphoblastic leukemia (ALL) and 8 sporadic acute myeloblastic leukemia (AML) cases from Tunisian population were included in this study. Informed consent was obtained from the patients, relevant family members (healthy relatives) or their legal guardian as required by the Helsinki Declaration.

DNA extraction

Genomic DNA extraction was performed on peripheral blood cells obtained during complete remission as defined by standard protocols of treatment. The EZ1 DNA tissue kit (Qiagen, Hilden, Germany) was used for DNA extraction according to the manufacturer's instructions. DNA was extracted from paraffin-embedded sections when no peripheral blood was available.

GATA2 sequencing

The entire coding regions of *GATA2* gene were amplified and sequenced. The primers used were exon 2F: CGGGACTG GTGCTCTTTCT; exon 2R: TTTTCAGCAGCTCG ATTCCT; exon 3-1F: GGGTTCCCTGTAGGGTCTGT; exon 3-1R: GTACTTGACGCCGTCCTTGT; exon 3-2F: ACTCTGGCTCCCACCTTTTC; exon 3-2R: GAGTCACT TCCCTCGCTTCA; exon 4F: GACTCCCTCCCGAG AACTTG; exon 4R: GCGTCTGCATTTGAAGGAGT; exon 5F: TTAGCCCTCCTTGACTGAGC; exon 5R: AGCCAAGCTGGATATTGTGG; exon 6F: CTATGAAG GTCGGGCACAAT; and exon 6R: GGTGGGGAACATTC ACAGTA. Purified PCR products were sequenced using the BigDye Terminator cycle sequencing ready reaction Kit v1.1 (Applied Biosystems—Foster City, USA) and loaded onto an ABI Prism 3500 sequencer (Applied Biosystems). The SeqScape software program v2.5 (Applied Biosystems) was used to search for new variants in sequencing products.

Mutational analysis

Allelic frequencies of the *GATA2* variants found were compared to those in general population as presented in the Exome aggregation consortium (ExAC), Cambridge, MA (URL: http://exac.broadinstitute.org).

PolyPhen-2 program was used to predict the impact of the obtained variants on the structure and function of GATA2 protein [11].

Results

The entire *GATA2* gene was sequenced in 119 hematological malignancies cases including 21 sporadic cases and belonging either to Tunisian or French populations. All identified *GATA2* gene variants are reported in (Table 1, Fig. 1). Their frequencies in general population as showed in ExAC database, and

Table 1 Screening results of the *GATA2* gene and alleles frequencies

	Variant	Protein	rs ID	PolyPhen2	Prediction	ExAC	Total allelic frequency	Frequency in familial cases	Frequency in sporadic cases
Exon 2	c.15C>G	p.Pro5Pro	rs1573858	−1.664	Polymorphism	0.6943	0.68907563	0.693877551	0.666666667
Exon 2	c.189C>T	p.Pro63Pro	rs763735447	−0.767	Disease causing	0.00002245	0.012605042	0.015306122	0
Intron2	c.229+38C>T	–	rs188629393	0.192	Polymorphism	0.007446	0.004201681	0	0.023809524
Exon 3	c.481C>G	p.Pro161Ala	rs34799090	2.414	Disease causing	0.009627	0.008403361	0.010204082	0
Exon 3	c.490G>A	p.Ala164Thr	rs2335052	0.464	Polymorphism	0.2056	0.130252101	0.147959184	0.047619048
Exon 3	c.564G>C	p.Thr188Thr	rs34870876	−1.483	Polymorphism	0.04238	0,046218487	0,051020408	0,023809524
Intron4	c.1017+49G>A	–	Unknown	−0.435	Polymorphism	0.000008257	0.004201681	0	0.023809524
Intron5	c.1018-19C>T	–	rs11708606	0.226	Polymorphism	0.1628	0.205882353	0.18877551	0.285714286
Exon 6	c.1187G>A	p.Arg396Gln	Unknown	5.811	Disease causing	NA	0.004201681	0	0.023809524
Exon 6	c.1233G>A	p.Ala411Ala	rs34172218	−3.324	Polymorphism	0.02224	0.021008403	0.025510204	0
Exon 6	c.1416G>A	p.Pro472Pro	rs376805544	−0.283	Disease causing	0.0002722	0.004201681	0.005102041	0

NA not available

their frequencies in the studied cohort are also presented. To predict the impact of each variant on GATA2 protein, in silico analysis was performed by PolyPhen-2 and the scores are shown in (Table 1).

Most of the identified variants in exons 2, 3, 6, and introns 4, 5 are known polymorphisms. However, one *p.Arg396Gln* variant in exon 6 was found at heterozygous level in one female Tunisian patient diagnosed with sporadic AML at the age of 38 and previously diagnosed with breast cancer. This variant is a known pathogenic mutation located in the second conserved Zinc-finger domain of the GATA2 protein. We also found a new *c.1017+49G>A* variant in intron 4 with unknown significance, which is carried by another 44-year-old Tunisian male patient diagnosed with sporadic AML.

Discussion

GATA2 gene encodes a zinc-finger transcription factor involved in the regulation of hematopoiesis. Mutations in this gene have been related to various hematological malignancies, particularly in MDS/AML familial aggregations [1, 6–8]. Several mutations, involving *GATA2* gene, were reported such as *p.Thr354Met* and *p.Thr355del* which may hamper the transactivation of target genes and impact the cellular differentiation and apoptosis. Most of *GATA2* mutations were associated to myeloid lineage such as AML and MDS which is characterized by a clonal disorder of hematopoietic stem cells and may progress to AML forms [12]. High levels of *GATA2* expression were observed in AML and were associated with poor prognosis [13]. Somatic mutations in *GATA2* have been reported basically among patients with chronic myeloid leukemia (CML).

Due to the increased incidence of *GATA2* mutations in several contexts of sporadic and familial hematological malignancies, we targeted this gene through a mutational analysis. Therefore, we aimed in this study to search for germline mutations by screening *GATA2* gene in 119 patients belonging to 98 independents Tunisian and French families diagnosed with hematological malignancies and 21 patients with sporadic acute leukemia.

Among all the identified variants (Table 1), only the *p.Arg396Gln* variant in exon 6 was found in one patient diagnosed with sporadic AML at the age of 38 and previously diagnosed with breast cancer. This variant has been previously reported in the literature as a disease causing mutation. In fact, this mutation is very common in familial hematological malignancies and particularly in the MonoMac syndrome [14]. The *p.Arg396Gln* variant is located in the second Zinc-finger domain of the GATA2 protein. This domain is highly conserved through evolution (Fig. 1). Identified mutations have been associated with leukemia and breast cancer [1]. This is observed in a sporadic AML case analyzed through this study.

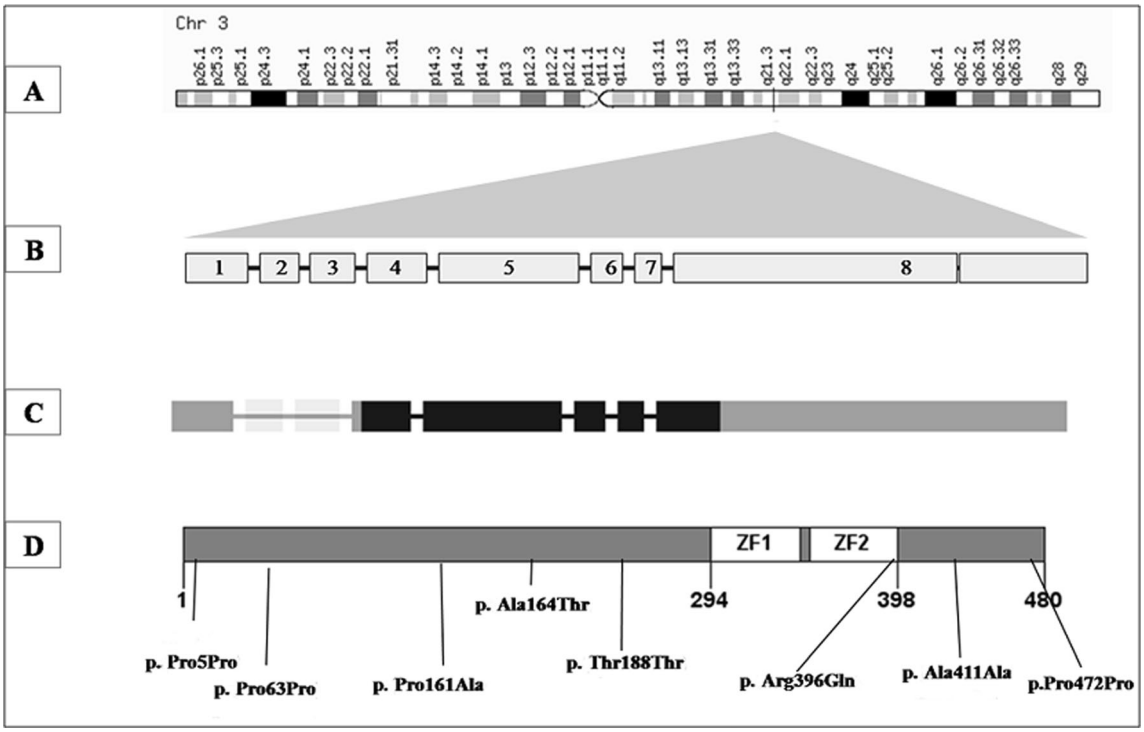

Fig. 1 Mutations distribution in *GATA2* gene. **a** Chromosomal location in 3q21.3. **b** *GATA2* gene structure. Exons are illustrated to scale. **c** *GATA 2* gene transcript (NM_032638.4). Coding exons are shown in *black*. **d** GATA2 protein structure containing 480 amino acids and the two Zing-finger domains (ZF1, ZF2). The mutations identified in our study are localized

The *p.Arg396Gln* mutation leads to decreased expression of the protein and loss of protein function which results in haploinsufficiency. In fact, this mutation hampers the DNA binding ability of the Zinc-finger domain of the protein, which impairs the activation of the *GATA2* promoter. The *p.Arg396Gln* mutation can cause disease in the heterozygous state as reported in the literature [14] and found in the patient described through this study.

We have identified another variant c.1017+49G>*A* in AML case without familial history. This variant with unknown significance is located in the intron 4 of *GATA2* gene. As indicated in the ExAC database, *c.1017+49G>A* variant is a very rare in the general population (ExAC frequency = 0.000008257). The sequence of the *GATA2* fourth intron is very conserved through evolution and it harbors the GATA-box-E-box composite element which is a regulatory element of transcription. Mutations in this intron result in haploinsufficiency of the protein and have been related to MonoMac Syndrome [2, 7, 15]. Thus, the *c.1017+49G>A* variant could have a deleterious impact on the GATA2 protein and should be more investigated.

The others *p.Pro5Pro (rs1573858)* and the *p.Ala164Thr (rs2335052)* variants found in this study have been identified while associating them with coronary artery disease [16]. The *p.Ala164Thr (rs2335052)* variant was also associated to reduce disease-free survival in colorectal cancer patients [17]. However, none of these variants were described in hematological malignancies cases. Concerning the *p.Ala411Ala* (rs34172218) and the *p.Pro472Pro* (rs376805544) variants identified in this study, both were detected only in familial aggregations of hematological malignancies and were totally absent in sporadic ALL and AML cases in our investigation. These two variants were not assigned to any pathology in literature.

Previous studies have identified other genes while associating them with hematological malignancies. In fact, *Gilliland*, *D*. *G* has suggested a multi-hit model in which many genes should be mutated to result in hematological malignancies [18]. Concomitant mutations to *GATA2* were described in literature involving majorly the *CEBPA* gene in sporadic AML, both genes act as transcription factors that function in the same differentiation pathway in AML and their alteration lead to leukemogenesis [19–22]. We have already screened several genes in our cohort in order to detect concomitant mutations in *TP53*, *IDH1*, *IDH2*, *JAK2*, *CBL*, *RUNX1*, *NPM1*, *ASXL1*, *ARL11*, and *CEBPA* [23–27] and *AML1*, *FAS*, *FASLG*, *PRF1*, *CASP8*, *CASP10*, *CRAF*, *BRAF*, *CBL*, *and YY1* (data not shown). A new *CEBPA* mutation *p.Gly242Ser* was reported in one familial case diagnosed with AML but no concomitant mutation with *GATA2* gene was observed [28].

In this study, we targeted for the first time, the entire *GATA 2* gene in a large series of 119 patients belonging to different ethnic populations; Tunisian and French, consisting of sporadic and familial hematological malignancies. Despite the wide

number of analyzed cases, we did not identify a deleterious variant in *GATA2* gene. The only *p.Arg396Gln* mutation found in one sporadic AML case highlights the involvement of *GATA2* gene in myeloid lineage malignancies majorly AML as was described in the literature. We did not report any germline *GATA2* mutations even in familial aggregations of MDS/AML. These findings underline the fact that *GATA2* mutations might be an important predisposing factor, but as a secondary genetic event, required for the development of overt malignant disease. Thus, other investigations should be carried out in order to identify the genetic factors underlying the hematological malignancies in the hole of the cohort. As hematological malignancies are very heterogeneous disorders, a large sequencing panels of the various genes involved in these pathologies are recommended.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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