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A novel frameshift mutation in *BLM* gene associated with high sister chromatid exchanges (SCE) in heterozygous family members

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Abstract The Bloom syndrome (BS) is an autosomic recessive disorder comprising a wide range of abnormalities, including stunted growth, immunodeficiency, sun sensitivity and increased frequency of various types of cancer. Bloom syndrome cells display a high level of genetic instability, including a 10-fold increase in the sister chromatid exchanges (SCE) level. Bloom syndrome arises through mutations in both alleles of the *BLM* gene, which was identified as a member of the RecQ helicase family. In this study, we screened a Tunisian family with three BS patients. Cytogenetic analysis showed several chromosomal aberrations, and an approximately 14-fold elevated SCE frequency in BS cells. A significant increase in SCE frequency was observed in some family members but not reaching the BS patients values, leading to suggest that this could be due to the heterozygous profile. Microsatellite genotyping using four fluorescent dye-labeled microsatellite markers revealed evidence of linkage to *BLM* locus and the healthy members, sharing higher SCE frequency, showed heterozygous haplotypes as expected. Additionally, the direct *BLM* gene sequencing identified a novel homozygous frameshift mutation c.3617–3619delAA (p.K1207fsX9) in BS patients and a heterozygous BLM mutation in the family members with higher SCE frequency. Our findings suggest that this latter mutation likely leads to a reduced BLM activity explaining the homologous recombination repair defect and, therefore, the increase in SCE. Based on the present data, the screening of this mutation could contribute to the rapid diagnosis of BS. The genetic confirmation of the mutation in *BLM* gene provides crucial information for genetic counseling and prenatal diagnosis.

Keywords Bloom syndrome · Sister chromatid exchange · *BLM* gene · *BLM* mutation

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Introduction

The Bloom syndrome (BS, MIM# 210900) is a rare human autosomic recessive disorder associated with growth retardation, immunodeficiency, sunlight sensitivity and an increased risk of malignancy at a young age [1–4]. Besides these clinical manifestations, the BS cells exhibit a number of cytogenetic abnormalities, including chromosome breakage, quadriradial chromatid interchanges, and especially a 10-fold elevated sister chromatid exchanges (SCEs) level, which is the hallmark of BS [2].

Bloom syndrome is caused by inactivating mutations in both copies of the *BLM* gene which is located on chromosome 15 at 15q26.1 and encodes a 1,417 amino acid

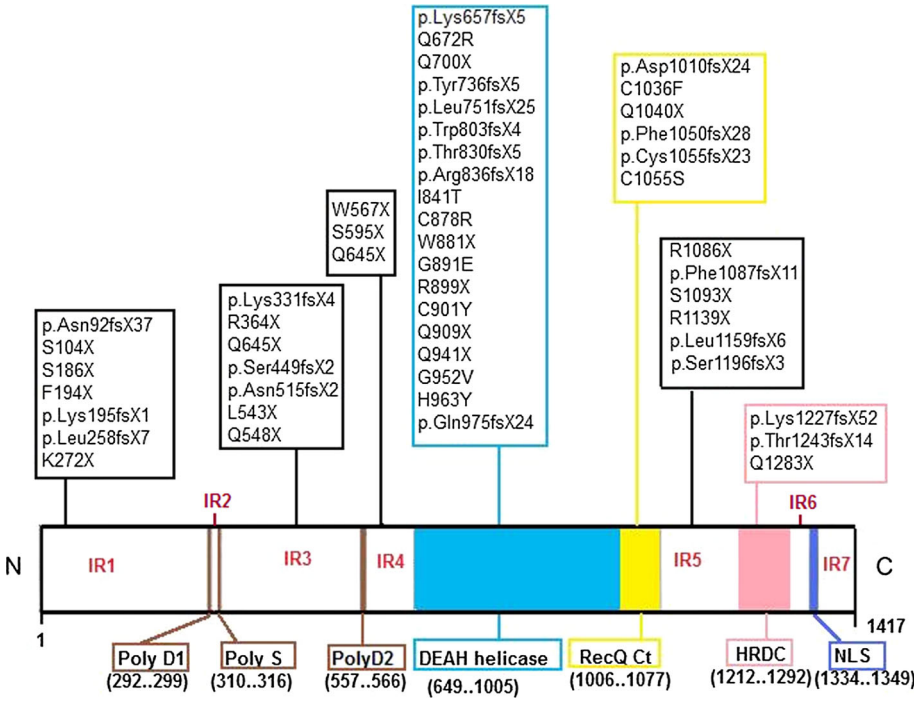

Fig. 1 Schematic depiction of distribution of the wild-type *BLM* protein and the identified mutations. *BLM* protein consists of seven domains, from the amino terminus poly-aspartate domain (Poly D1), poly-serine domain (Poly S), poly-aspartate domain (Poly D2), DEAH helicase domain (DEAH), RecQ helicase C-terminal domain (RecQCt), helicase and RNase D C-terminal domain (HRDC), and nuclear localization signals (NLS). *IR* intervening region. All currently known mutations are presented in *boxes* according http://bioinf.uta.fi/BLMbase/

protein (159 kDa), homologous to the RecQ subfamily of DExH box-containing DNA and RNA helicases [5]. BLM protein consists of seven domains: The amino terminus poly-aspartate domain (Poly D1), poly-serine domain (Poly S), poly-aspartate domain (Poly D2), DEAH helicase domain (DEAH) [6], RecQ helicase C-terminal domain (RecQCt) [6], helicase and RNase D C-terminal domain (HRDC) [7], and nuclear localization signals (NLS) [8] (Fig. 1).

Like other RecQ family helicases, BLM interacts physically and functionally with numerous proteins in the cell to perform its diverse functions [9–13]. BLM helicase activity is necessary for genomic instability correction. Thus, several in vitro and in vivo studies demonstrated that BLM unwinds the canonical Watson–Crick duplex, and recognizes and disrupts alternative DNA structures including the Holliday junctions, the triple helices and the highly stable G-quadruplex [14–19].

Up to date, a number of nonsense or frameshift mutations and missense mutations are found in the *BLM* gene of BS patients, according to the previous reports [20–22]. Frameshift mutations are found along the entire length of *BLM* gene, whereas all missense mutations affect the DEAH helicase or the RecQ-CT domains (Fig. 1).

In the present study, we performed clinical, cytogenetic, microsatellite genotyping and mutational analyses on a Tunisian consanguineous family with BS. We identified several chromosomal aberrations (CA) and an approximately 14 fold-elevated SCE frequency in BS cells associated with a novel homozygous frameshift mutation c.3617–3619delAA (p.K1207fsX9). Heterozygous *BLM* mutation was found to be associated with an SCE increase in the healthy family members, a finding which has not been reported elsewhere.

Materials and methods

Studied subjects

A consanguineous family (Is.) (12 members) from Sidi Bouzid (South of Tunisia) with three affected males (Fig. 2) referred to the Dermatology Department, Hedi Chaker Hospital, Sfax, Tunisia. The age of the affected individuals ranged from 27 to 38 years. For the present study, fifty (50) control individuals were also tested. A questionnaire on clinical information and family history was drawn up. Informed consent was obtained from all the family members and control individuals in accordance with the ethic committee of the University Hospital of Sfax, Tunisia.

CA and SCE analysis

Separate lymphocyte cultures were set up for CA and SCEs assays. A detailed description of CA and SCE assays was reported in our previous studies [22, 23].

Microsatellite genotyping

Four (4) fluorescent dye-labeled microsatellite markers (D15S996, D15S130, D15S127 and D15S158) spanning

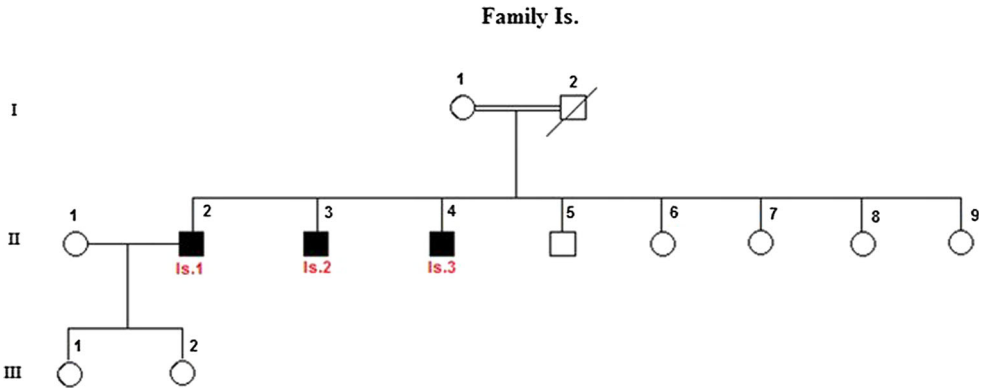

Fig. 2 Pedigree of the Bloom syndrome family; males and females are represented by *squares* and *circles*, respectively

the BLM locus (15q26.1) were selected on the basis of their map position and heterozygosity coefficient. The distances between the two close markers (D15S996 and D15S127) and the BLM gene are 39 and 273 Kb, respectively. The markers were genotyped for the family members after DNA extraction according to the phenol–chloroform protocol [24]. True Allele PCR Premix (Applied Biosystems, Foster City, CA, USA) was used for PCR reactions according to the manufacturer's instructions. Fluorescent labelled alleles were analysed on an ABI PRISM 3100-Avant automated Genetic Analyser (Applied Biosystems). The genotypes were determined using GenScan software (Applied Biosystems). The construction of haplotypes in genotyped members was done regardless of the individual's affection status.

PCR amplification of the *BLM* gene

Mutational analysis was performed by PCR amplification of each of the 21 encoding exons of *BLM* gene and the intron–exon boundaries using appropriate primers chosen so that at least 80 bp of flanking intronic sequences were readable. Primers were shown in our previous study [22]. Intronic sequences containing known polymorphic sites were avoided to prevent false-negatives resulting from the amplification of single allele. PCR amplification of each exon was performed in a thermal cycler (Gene Amp PCR system 9700, Applied Biosystem) [22].

Sequencing of the *BLM* gene and bioinformatics analysis

PCR product was purified using exonuclease before sequencing. Each exon was sequenced on both strands. The region containing putative novel variations was sequenced twice on both strands to exclude that they were PCR artefacts. Direct sequencing of PCR products was performed in an ABI PRISM 3100-Avant automated DNA sequencer using the Big Dye Terminator Cycle Sequencing reaction kit v1.1 (Applied Biosystems). The identified mutation of the *BLM* gene was investigated by direct sequencing in 50 Tunisian healthy individuals. The BLAST homology searches were performed using NCBI website (http://www.ncbi.nlm.nih.gov/).

Results

A consanguineous family (12 members) from Sidi Bouzid (South of Tunisia) with three affected males (Fig. 2) referred to the Dermatology Department, Hedi Chaker Hospital, Sfax, Tunisia.

Clinical characteristics of BS patients

The physical and biological data collected during the follow-up are summarized in Table 1. The medical history revealed a proportionate dwarfism, small stature, reduced head circumference, telangiectatic erythema with sun sensitivity (see supplementary Figure). According to the questionnaire data, the BS patients had a smoking habit of more than 10 years. However, fortunately, none of the patients had cancer at their most recent follow-up visit when they were between 5 and 38 years old.

Cytogenetic analysis

The results indicated multiple CA in the BS cells such as chromatid breaks, chromosome breaks, acentrics, dicentrics, gaps, fragile sites, and especially telomeric associations, which are characteristic of BS cells (Fig. 3). Furthermore, an approximately 14-fold increase in SCE levels was found in BS patients compared to the control group (7.34 ± 2.31) ($p < 0.001$) (Table 2). The mean frequencies of SCE per cell were 96.39 ± 18.40, 113.40 ± 22.32, and 110.50 ± 12.52 in BS patients Is.1, Is.2 and Is.3, respectively (Table 2). Interestingly, an unexpected heterogeneity was observed in the mean

Table 1 Physical and biological data collected during the follow up for the three affected males

Patient	Age[a] (years)	Height[a] cm (SD)	Head circumference[a] cm (SD)	Face	Associated skin lesions	Sunlight sensitivity	Associated clinical anomalies	Cancer[a]	Immune defect
Is.1	38	152(−2SD)	53(−2SD)	Narrow face Malar hypoplasia Small mandibles	Café au lait spots Achromic patches	Vesicles bullae	Ureter duplication Brachyphalangea kyphosis Testicular atrophy	–	↓ IgA ↓ IgM ↑ FSH
Is.2	36	152(−1SD)	52 (−4SD)	Narrow face	Café au lait spots Achromic patches Ectropion Loss of eyelashes	Vesicles bullae	Moderate mental retardation	–	↓ IgA ↓ IgM
Is.3	27	150(−1SD)	50 (−2SD)	Narrow face	Achromic patches	In summer	Ureter duplication	–	↓ IgA ↓ IgM ↓ IgG

[a] At the least follow-up

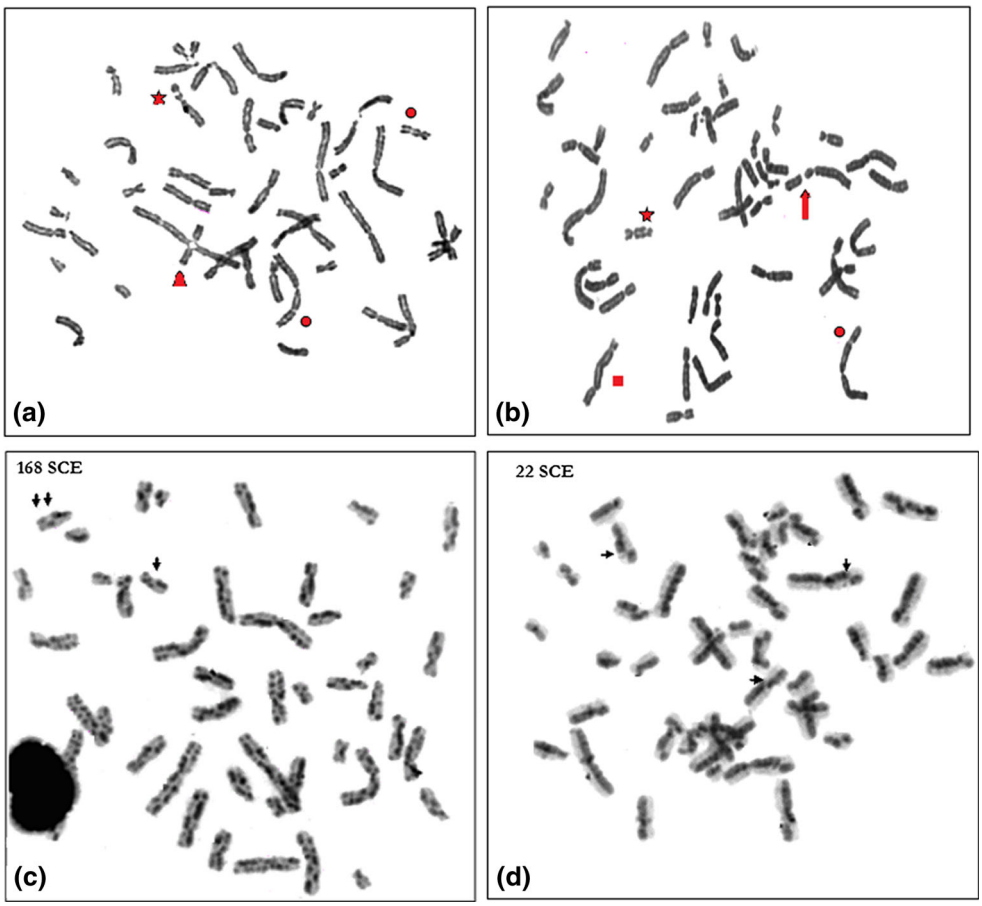

Fig. 3 Metaphases observed with CA assay (**a, b**) and SCE assay (**c, d**). CA are represented with the following red symbols: Chromatid breaks, *stars*; acentric fragment, *bold arrow*; dicentric chromosome, *square*; fragiles sites, *circles*; telomeric associations, *triangles*. Three of 168 and 22 SCE are represented by *black arrows* in cells from a BS patient (**c**) and a healthy brother (**d**), respectively

Table 2 The range and mean frequencies of SCE in BS family and control group

Subject	Range of SCE number	Mean of SCE ± SD
BS family I.1 (Mother)	8–20	10.61 ± 2.10*
II.1	5–15	7.79 ± 1.34
II.2 (Patient Is.1)	80–120	96.39 ± 18.40**
II.3 (Patient Is.2)	85–170	113.4 ± 22.32**
II.4 (Patient Is.3)	90–146	110.5 ± 12.52**
II.5 (Brother)	7–22	10.49 ± 3.47*
II.6	6–17	8.65 ± 3.29
II.7	6–15	6.74 ± 2.12
II.8	6–12	6.85 ± 1.20
II.9	7–17	8.12 ± 1.67
III.1 (Daughter of Is.1)	8–21	11.40 ± 5.23*
III.2 (Daughter of Is.1)	7–19	10.67 ± 3.76*
Control group (50)	5–15	7.34 ± 2.31

SD standard deviation

* $p < 0.05$; ** $p < 0.001$ (compared *vs* control group)

frequency of SCE within the healthy family members. So, compared with control group, a significant increase was found in the mother (I.1) (10.61 ± 2.10 SCE/cell), in the healthy brother (II.5) (10.49 ± 3.47 SCE/cell) (Fig. 3), and in the two daughters (III.1 and III.2) of the patient Is.1 (11.40 ± 5.23 SCE/cell and 10.67 ± 3.76 SCE/cell) ($p < 0.05$).

Microsatellite genotyping

According to the heterogeneity of SCE frequencies among the healthy family and since four of the nine members shared a significantly higher SCE frequency, we hypothesized that these members might be heterozygous for *BLM* mutation. Before *BLM* gene sequencing, we performed a haplotype analysis on all the family members using four fluorescent dye-labeled polymorphic microsatellite markers covering all *BLM* locus. This analysis revealed evidence of linkage to *BLM* mapping in chromosome 15q. Bloom syndrome patients showed a homozygous haplotype for alleles 194, 145, and 84 bp of D15S996, D15S127, and D15S158 microsatellite markers, respectively (Fig. 4). As expected by SCE data, heterozygous haplotypes were observed in members I.1, II.5, III.1 and III.2 (Fig. 4).

BLM gene mutational analysis

To further strengthen this hypothesis and confirm the haplotypes data, we performed *BLM* gene sequencing for all family members. The entire *BLM* gene coding sequence of (exon 2–22) was, therefore, analysed, including all intron–exon junctions and at least 80 bp of intronic sequence flank each exon. A novel homozygous mutation c.3617–3619delAA was identified in BS patients as predicted by the consanguinity of their parents and the markers haplotype (Fig. 4). This mutation occurred within exon 19 of the *BLM* gene and predicted to generate a premature termination codon (p.K1207fsX9). The healthy members (I.1, II.5, III.1 and III.2) sharing higher SCE frequencies were found to be heterozygous for the *BLM* mutation; while, it was absent in the rest of family members and the 50 healthy controls (Fig. 4).

Discussion

Our study reports clinical, cytogenetic, microsatellite and mutational analyses on three patients, belonging to a consanguineous Tunisian family, with typical BS clinical features. Cytogenetic analysis by SCE and CA assays confirmed the suspected diagnosis of the patients. Indeed, in line with previous reports, BS cells display chromosome breaks, spontaneous symmetric quadriradial interchanges and an increase in SCE levels [2, 22, 25]. However, in our study, the SCE mean frequencies in BS patients seemed to be higher than those reported in previous study [2, 22, 25]. It was an approximately 14-fold higher than control frequency especially, in patients Is.2 and Is.3. This finding could be due to the smoking effect, according to our previous study [23] that reported an increase of SCE frequency in higher smokers (smoking habit >10 years).

In somatic cells, SCEs are mediated by homologous recombination (HR) [26]. It has been reported in Sonoda study that HR uses nascent sister chromatid to repair potentially lethal DNA lesions accompanying replication, which might explain the lethality or tumorigenic potential associated with defects in HR or HR-associated proteins [26]. In BS cells, the BLM protein unwinds DNA structures mimicking replication forks and HR intermediates in combination with topoisomerase IIIα, RMI1 and RMI2 (BTR complex) to dissolve double Holliday junctions [17, 27–30].

In addition, it has been reported that BLM interacts with RAD51 and can efficiently disrupt D-loops and might, therefore, act to prevent inappropriate template usage during HR [31–33]. BLM might suppress SCEs through the dismantling of the D-loops to promote synthesis-dependent strand annealing, a pathway of HR that prevents crossovers formation and thus SCEs [32, 33]. Therefore, the BS cells defective for BLM protein could engender an elevated frequency of homologous and non-homologous tri and quadriradial chromosomes and SCEs.

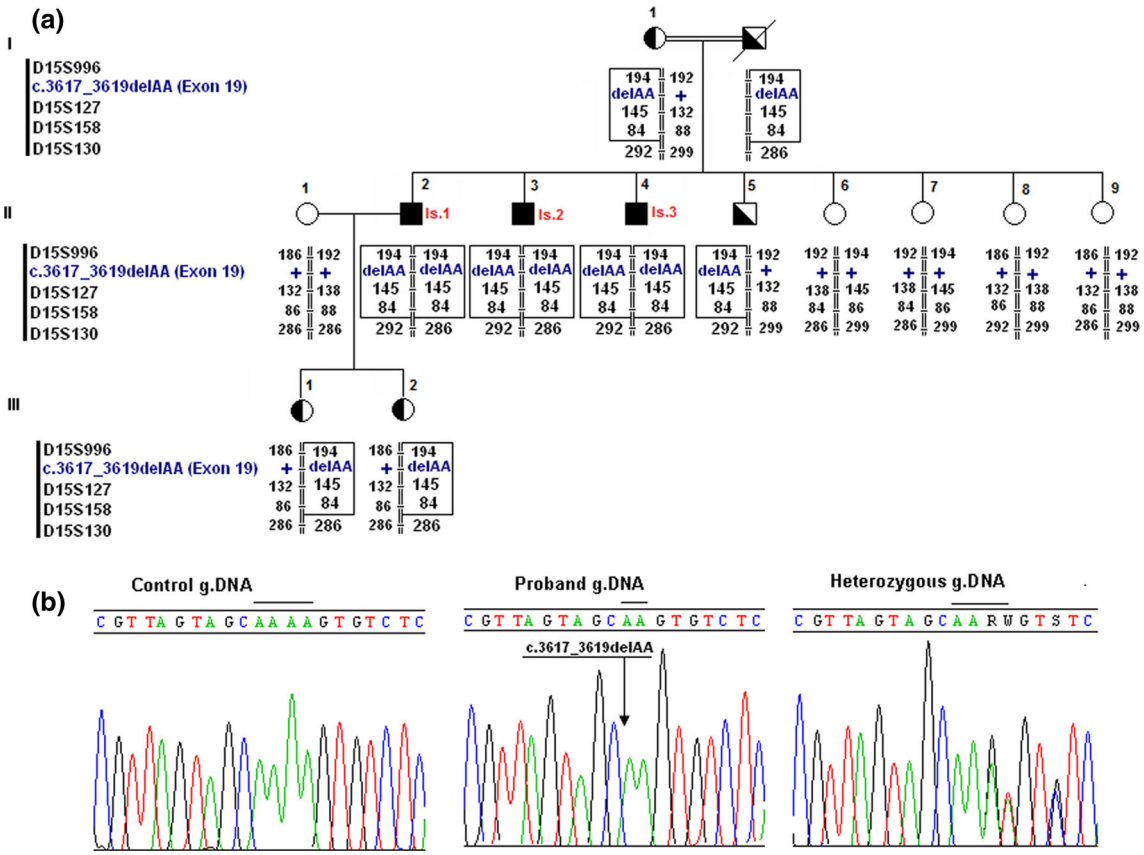

Fig. 4 Microsatellite genotyping and mutation analyses. **a** Pedigree of the family showing the segregation of BLM haplotypes and the inheritance of the c.3617–3619delAA mutation. The distances between the two markers D15S996 and D15S127 and the *BLM* gene are 273 and 39 Kb, respectively. The part of haplotypes not boxed is non-informative for all patients and their relatives. +: Presence of c.3617–3619AA. **b** Sequence chromatograms showing the presence of the new homozygous c.3617–3619delAA mutation in exon 19 in the studied patients (proband) (*at the middle*, nucleotides deletions are indicated by an *arrow*), the heterozygous mutation profile in some healthy family members (*at the right*) and its absence in a control (*at the left*). Mutation profiles in family members are represented with the following symbols: homozygous *BLM* mutation, *black squares*; heterozygous *BLM* mutation, *half squares* and *half circles* in black; homozygous wild-type; *white circles*

Interestingly, in this study, an unexpected heterogeneity in SCE frequency in the healthy family members was observed. So, a significant increase in SCE frequency was noted in some family members, but did not lead the BS patient's values. This finding has not been observed elsewhere and, therefore, leads us to suggest that this could be due to the heterozygous profile of *BLM* gene since these members were non-smokers and without any indication of previous occupational/environmental genotoxic exposure or other agents suspicious of genotoxicity which could increase their SCE rates.

To confirm our hypothesis, we performed a haplotype analysis on all the family members using microsatellite markers covering the *BLM* gene. This analysis revealed evidence for linkage to *BLM* mapping to chromosome 15, and, therefore a homozygous haplotype was observed in BS patients, which supported our hypothesis that the healthy members sharing higher SCE frequency might be heterozygous for *BLM* gene. To strengthen this suggestion, we investigated a direct *BLM* gene sequencing.

Mutational analysis of *BLM* gene revealed a novel homozygous mutation c.3617–3619delAA in exon 19 of BS patients; however, as expected by haplotype analysis, a heterozygous *BLM* mutation was found in the healthy family members sharing higher SCE frequency. This suggests that this heterozygous mutation might reduce BLM activities explaining the HR defects and, therefore, the increase in SCE. This is in disagreement with Ellis' study showing normal SCE rates in BS cells with at least one non mutant *BLM* allele [34].

The c.3617–3619delAA mutation seemed to be specific to the Tunisian population, consistent with the considerable mutations diversity affecting the *BLM* gene (http://bioinf.uta.fi/BLMbase/). The c.3617–3619delAA mutation introduced a premature stop codon (p.K1207fsX9) in the intervening region 5 (IR5) situated between RecQCt

domain and HRDC domain. As a result, this small deletion likely leads to a truncated protein containing an intact DEAH helicase and the RecQ-CT domains located in the first 1,077 amino acids [6] but lacking the HRDC domain situated between amino acids 1,212–1,292 [7] and NLS domain located between amino acids 1,334–1,349 [8] in the C-terminal region of *BLM* protein (see Fig. 1).

The C-terminus may confer on BLM protein the ability to recognize and bind abnormal DNA structures such as quadruplexes [35]. It has been reported in Wu's studies that C-terminus deletions have a stronger negative effect on genomic stability than those occurring in N-terminus domain and that the mutant proteins can be incorporated into normal BLM-containing complexes and inhibit their function or regulation [36]. Helicase and RNase D C-terminal domain residing in the C-terminal region of BLM protein [7], is a critical determinant of the dissolution function of double Holliday junctions by the *BLM*–Topoisomerase IIIa complex [37]. In addition, Killoran (2006) reported that the HRDC domain could independently bind DNA and, consequently, the RecQ helicases containing mutations in the HRDC domain have altered DNA structure specific binding and unwinding properties [38]. Recently, Amor Guéret group (2008) identified a homozygous frameshift mutation (p.Ser1196fsX3) in the IR5 domain (Fig. 1), leading to a truncated BLM protein upstream to the HRDC domain; however, no data are available for the correlation between heterozygous mutation and cell phenotype [21].

Overall, in this study, we described a higher chromosomal instability in Tunisian BS patients associated with a novel homozygous fameshift mutation leading to the deletion of a BLM protein upstream to the HRDC domain. Additionally, we established that the heterozygous profile of this mutation seemed to be associated with BLM defects and higher SCE frequency in healthy family members. This identification may allow a genetic counselling in the relatives of this family and an antenatal diagnosis which is now possible.

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Conflict of interest We declare that we have no any actual or potential conflict of interest, including financial, personal or any relationships with other people or organizations concerning this work.

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