Received: 7 December 2021 | Revised: 3 February 2022 | Accepted: 23 February 2022

DOI: 10.1111/cge.14125

CLINICAL GENETICS WILEY

SHORT REPORT

Phenotypic spectrum of *BLM*- and *RMI1*-related Bloom syndrome

Ipek Ilgin Gönenc[1] | Nursel H. Elcioglu[2,3] | Carolina Martinez Grijalva[1] | Seda Aras[4] | Nadine Großmann[1] | Inka Praulich[1] | Janine Altmüller[5,6,7] | Silke Kaulfuß[1] | Yun Li[1] | Peter Nürnberg[5,8] | Peter Burfeind[1] | Gökhan Yigit[1,9] | Bernd Wollnik[1,9,10]

[1]Institute of Human Genetics, University Medical Center Göttingen, Göttingen, Germany

[2]Department of Pediatric Genetics, Marmara University Medical School, Istanbul, Turkey

[3]Eastern Mediterranean University School of Medicine, Cyprus, Mersin 10, Turkey

[4]Department of Pediatric Haematology and Oncology, Marmara University Medical School, Istanbul, Turkey

[5]Cologne Center for Genomics (CCG), University of Cologne, Cologne, Germany

[6]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Core Facility Genomics, Berlin, Germany

[7]Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

[8]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Faculty of Medicine, University Hospital Cologne, Cologne, Germany

[9]German Center for Cardiovascular Research (DZHK), partner site Göttingen, Göttingen, Germany

[10]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Göttingen, Germany

Correspondence
Bernd Wollnik, Institute of Human Genetics, University Medical Center Göttingen, Heinrich-Düker-Weg 12, 37073 Göttingen, Germany.
Email: bernd.wollnik@med.uni-goettingen.de

Funding information
Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Research Group FOR 2800 "Chromosome Instability: Cross-talk of DNA replication stress and mitotic dysfunction", SP5 and SPZ; German Center for Cardiovascular Research (DZHK, partner site Göttingen); Germany's Excellence Strategy, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC; EXC 2067/1-390729940)

Abstract

Bloom syndrome (BS) is an autosomal recessive disorder with characteristic clinical features of primary microcephaly, growth deficiency, cancer predisposition, and immunodeficiency. Here, we report the clinical and molecular findings of eight patients from six families diagnosed with BS. We identified causative pathogenic variants in all families including three different variants in *BLM* and one variant in *RMI1*. The homozygous c.581_582delTT;p.Phe194* and c.3164G>C;p.Cys1055Ser variants in *BLM* have already been reported in BS patients, while the c.572_573delGA;p.Arg191Lysfs*4 variant is novel. Additionally, we present the detailed clinical characteristics of two cases with BS in which we previously identified the biallelic loss-of-function variant c.1255_1259delAAGAA;p.Lys419Leufs*5 in *RMI1*. All BS patients had primary microcephaly, intrauterine growth delay, and short stature, presenting the phenotypic hallmarks of BS. However, skin lesions and upper airway infections were observed only in some of the patients. Overall, patients with pathogenic *BLM* variants had a more severe BS phenotype compared to patients carrying the pathogenic variants in *RMI1*, especially in terms of immunodeficiency, which should be considered as one of the most important phenotypic characteristics of BS.

KEYWORDS
BLM gene, Bloom syndrome, growth deficiency, immunodeficiency, *RMI1* gene

1 | INTRODUCTION

Bloom syndrome (BS, MIM 210900) is a rare congenital disorder presenting with primary microcephaly, growth deficiency, photosensitivity, cancer predisposition, and immunodeficiency.[1] BS is inherited autosomal recessively[2] and mainly caused by biallelic loss-of-function (LoF) variants in the *BLM* gene.[3] *BLM* encodes the BLM helicase, which has essential roles in the maintenance of genomic stability.[4] Accordingly, cells from BS patients show high levels of chromosome aberrations and mitotic defects, which overall contribute to the pathogenesis of the disease.[5,6] As an evidence of genomic instability, BS patient cells show elevated rates of sister chromatid exchange (SCE),[7] which is used as a cytogenetics test in the diagnosis of BS when molecular diagnostic tools are limited.

The BLM helicase forms part of a protein complex, known as the BTRR complex, which additionally includes the topoisomerase III alpha (TOP3A, MIM 601243) and the RecQ-mediated genome instability protein 1 (RMI1, MIM 610404) and 2 (RMI2, MIM 612426).[8–10] The BTRR complex dissolves DNA intermediates which occur during DNA replication and repair processes.[11,12] Apart from pathogenic variants in *BLM*, pathogenic variants in the other three members of the BTRR complex have been recently linked to BS.[3,13,14] The phenotype of patients with biallelic *TOP3A* variants was similar to those of patients with pathogenic *BLM* variants in terms of primary microcephaly and growth retardation.[2,13] However, dilated cardiomyopathy, which is not a typical characteristic of BS, was also reported in patients with pathogenic *TOP3A* variants.[13] Another study reported a large homozygous deletion covering *RMI2* to be associated with a BS phenotype and the diagnosis was confirmed by increased SCE rates, although the clinical features were reported as much milder than the classic BS phenotype.[14]

Here, we report a patient cohort of eight individuals presenting with main characteristics of BS phenotype. We identified one novel and two previously described biallelic pathogenic variants in *BLM* in six individuals and defined the clinical phenotype of patients with a biallelic LoF variant in *RMI1* that has recently been reported.[13] We further describe the molecular and clinical findings of the patients and compare the phenotypic expression.

2 | MATERIALS AND METHODS

All subjects or their legal representatives gave written informed consent to the molecular genetic analyses and the publication of the results. This study was performed according to the Declaration of Helsinki protocol and approved by the local institutional review board (University Medical Center Göttingen, Germany).

Genomic DNA samples were screened for variants in *BLM* (NM_000057.4) and *RMI1* (NM_024945.3) using specific primers and subsequent Sanger sequencing of amplified DNA (Supporting Information).

WES analysis was done for two patients. DNA samples were enriched and sequenced on an Illumina HiSeq4000 sequencer. The variants were analyzed using the "Varbank" pipeline from Cologne Center for Genomics (CCG). Filtering criteria are given in the Supporting Information. The resulting variants were considered as candidates when the phenotype–genotype link was built.

3 | RESULTS

3.1 | Mutational spectrum of Bloom syndrome patients

The molecular analyses revealed three distinct pathogenic variants in *BLM* in six patients (Figure 1A and Table 1). Using WES, we identified a novel, possible pathogenic variant, c.572_573delGA;p.Arg191Lysfs*4, in *BLM* in patient F1/II-4, which we confirmed by subsequent Sanger sequencing. By using a custom-designed multigene panel (Table S1), we were able to identify this novel *BLM* variant in homozygous state in a second patient with BS (F2/II-2; Table 2). Consanguinity was reported for both parents of family 1. In family 2, parental consanguinity was not reported, but both parents were originating from the same village with less than 1000 inhabitants. A homozygous variant (c.581_582delTT;p.Phe194*) in *BLM* was identified in patients F3/II-2 and F4/II-2 by WES and Sanger sequencing, respectively. This variant has been reported previously.[15] Furthermore, molecular investigation of *BLM* revealed homozygosity for the missense variant (c.3164G>C;p.Cys1055Ser) in two siblings (F5/II-2 and F5/II-3) whose consanguine parents were heterozygous carriers. All identified variants were confirmed by Sanger sequencing and segregation analysis on all parents was carried out upon detection of a sequence variant using the same sequencing approach (Figure 1B).

Additionally, we previously performed WES on the DNA of two *BLM*-variant negative patients, both born to consanguineous parents and presenting with a BS-like phenotype.[13] WES revealed a single truncating variant in *RMI1* which was present in a homozygous state in both patients.[13] No other variant was detected which could explain the disease phenotype.

3.2 | Clinical findings in Bloom syndrome patients

We reinvestigated the clinical features of our patients to determine the spectrum of *BLM*- and *RMI1*-associated phenotypes. All index

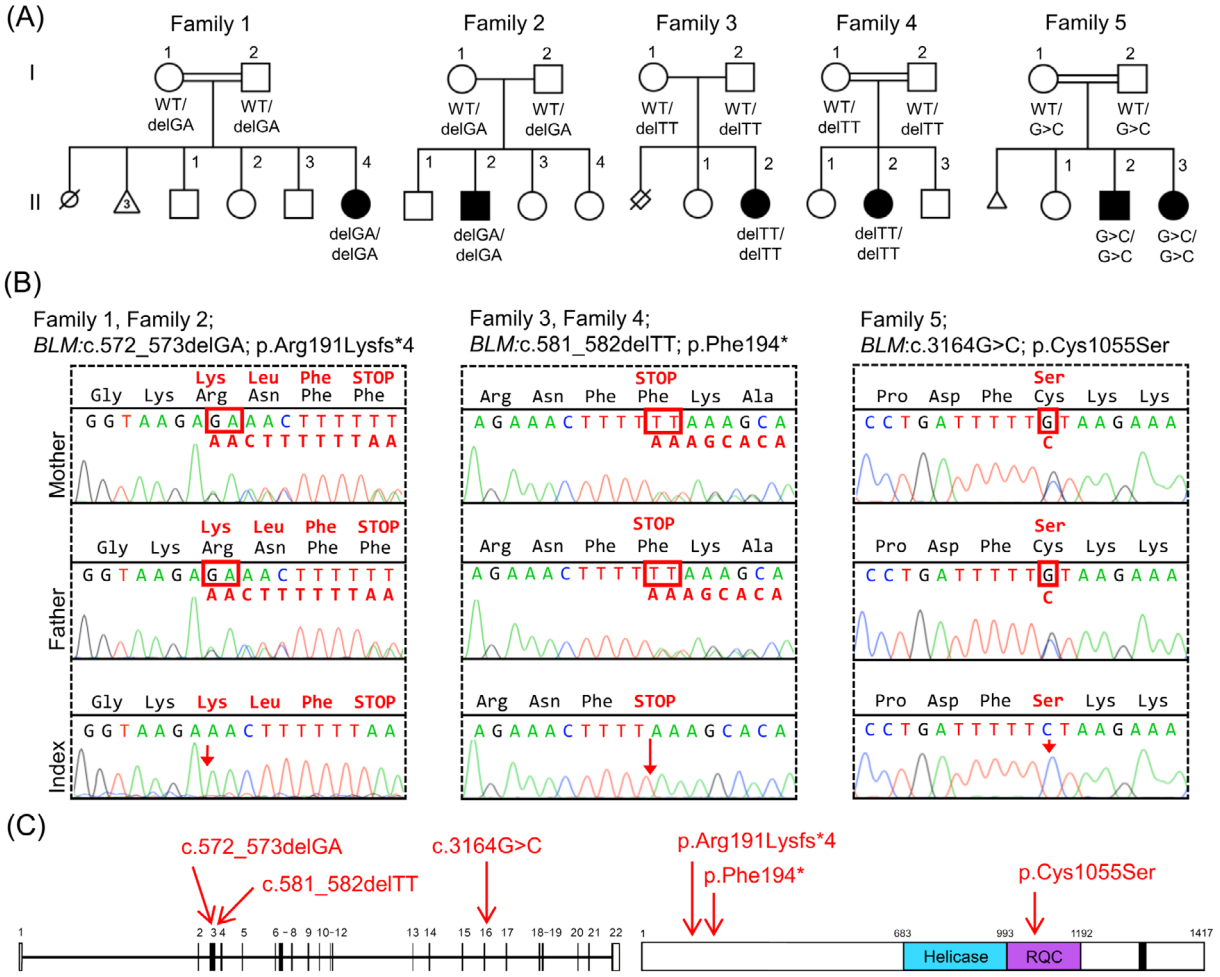

FIGURE 1 Pedigrees and the molecular characterization of pathogenic variants detected in *BLM*. (A) Pedigrees of five unrelated families with disease-causing variants in *BLM* suggesting an autosomal-recessive inheritance pattern. (B) Electropherograms of the identified variants in *BLM*. Affected siblings in each family carry disease-causing variants in *BLM* in the homozygous state (lower panels) while non-affected parents are heterozygous for identified *BLM* variants (upper and middle panels). (C) Positions of the identified pathogenic variants are shown in reference to the schematic representation of the *BLM* gene (left) and BLM protein (right). Exons are shown as dark boxes, 5′- and 3′-UTRs as open boxes. Conserved domains of the BLM protein are shown in blue (helicase domain), purple (RecQ C-terminal domain), and black (nuclear localization signal) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Pathogenic variants identified in *BLM* and *RMI1* in patients with Bloom syndrome phenotype

Family	Patient	Gene	Exon	Variant (homozygous)	Predicted protein outcome	Parental consanguinity	Allele frequency[a]
1	F1/II-4	*BLM*	3	c.572_573delGA	p.Arg191Lysfs*4	+	–
2	F2/II-2	*BLM*	3	c.572_573delGA	p.Arg191Lysfs*4	-[b]	–
3	F3/II-2	*BLM*	3	c.581_582delTT	p.Phe194*	–	0.000008
4	F4/II-2	*BLM*	3	c.581_582delTT	p.Phe194*	+	0.000008
5	F5/II-2	*BLM*	16	c.3164G>C	p.Cys1055Ser	+	0.000017
	F5/II-3	*BLM*	16	c.3164G>C	p.Cys1055Ser	+	0.000017
6[c]	F6/1	*RMI1*	3	c.1255_1259delAAGAA	p.Lys419Leufs*5	+	0.000008
	F6/2	*RMI1*	3	c.1255_1259delAAGAA	p.Lys419Leufs*5	+	0.000008

Note: Transcript numbers are as follows: NM_000057.4 for *BLM* and NM_024945.3 for *RMI1*.

[a]The allele frequency is taken from ExAC database.

[b]The parents are from the same village with less than 1000 inhabitants.

[c]Patients from this family with pathogenic variants in *RMI1* have been previously reported.[13]

TABLE 2 Clinical findings of patients with pathogenic variants in the *BLM* and *RMI1* genes

	BLM						*RMI1*	
Patient ID	F1/II-4	F2/II-2	F3/II-2	F4/II-2	F5/II-2[b]	F5/II-3	F6/1[a]	F6/2[a]
Gender	F	M	F	F	M	F	F	F
Age at exam (years)	$16^{3/12}$	16	$3^{10/12}$	$13^{6/12}$	$8^{2/12}$	$1^{7/12}$	7	13
Pre- and postnatal microcephaly	+	+	+	+	+	+	+	+
Intrauterine growth retardation	+	+	+	+	n/a	+	+	+
Length at birth *z*-score (SD)	−4.3	n/a	n/a	N	N	N	n/a	n/a
Weight at birth *z*-score (SD)	−5	N	n/a	−3,3	−2,7	N	−1,7	−3,1
OFC at birth *z*-score (SD)	n/a	n/a	n/a	n/a	n/a	−3,2	n/a	n/a
Postnatal growth deficiency	+	+	+	+	+	+	+	+
Height *z*-score (SD)	−3,9	−4,6	−4,2	−6	−1,8	−2,3	−4	−2,9
Weight *z*-score (SD)	−5,5	−4,7	−3,2	−4,9	−3,6	−4,2	−3,8	−0,9
OFC *z*-score (SD)	−6	−5,1	−4,7	−7,6	−3,1	−5,1	−5	−5
Short stature	+	+	+	+	+	+	+	+
Narrow face	−	+	−	+	+	−	−	−
Skin lesions	−	+	+	−	+	+	−	−
Café-au-lait spots	−	+	+	+	+	+	−	−
Immunodeficiency	+	+	+	+	+	+	-[c]	−
IgG	N	low	low	low	n/a	low	n/a	n/a
IgA	low	low	low	n/a	low	low	n/a	n/a
IgM	low	low	low	n/a	low	low	n/a	n/a
IgG1	n/a	N	low	n/a	n/a	N	n/a	n/a
Recurrent infections	−	+	−	−	−	+	−	−
Upper airway/pulmonary infections	+	+	−	+	+	+	−	−

Note: The following abbreviations are used: M, male; F, female; n/a, data not available; N, normal.
[a]The two individuals carrying this pathogenic variant in this gene were previously reported.[13]
[b]Wilms' tumor was detected and operated at the age of 5.
[c]Immunoglobulin levels were not specified for this patient, but reported to be in normal range.

patients suffered from intrauterine growth deficiency and had a small head circumference. The most severe case of growth deficiency and microcephaly was observed in patient F4/II-2 (Table 2). In addition, growth hormones had been administered to F1/II-4, but treatment was unresponsive. A narrow face, sunlight sensitivity, and café-au-lait spots were observed in some but not all of the patients with pathogenic variants in *BLM*. On the other hand, sunlight-sensitive skin lesions, which are considered as a major phenotypic characteristic of BS, were present only in two of our *BLM*-mutated patients (F2/II-2 and F3/II-2). Interestingly, neither of the patients with pathogenic variants in *RMI1* showed any dermatological characteristics of BS.

All patients with biallelic pathogenic variants in the *BLM* gene had low immunoglobulin levels (Table 2). F5/II-2 experienced lower respiratory system infections each year, and F1/II-4 had upper respiratory system infections three to four times each winter. In contrast, neither of the patients carrying the pathogenic variant in *RMI1* had a history of recurrent (respiratory) infections. Serum immunoglobulin levels for patient F6/1 at the age of 7 were in normal range, testing for patient F6/2 was not performed.

4 | DISCUSSION

In rare and ultra-rare diseases, data on the phenotypic and mutational spectrum as well as possible phenotype–genotype correlations are hard to be obtained due to the limited number of affected cases. Although BS was described in 1954,[16] the most comprehensive patient cohort was reported in 2007 consisting of 134 BS patients.[3] Yet, nine patients lacked a disease-causing variant in *BLM*, indicating a likely genetic heterogeneity.[3,6] In this study, we report eight individuals diagnosed with BS. The molecular analyses revealed one novel and two known LoF variants in *BLM*. Moreover, we included a previously described truncating variant in *RMI1* into our study.[13] Our molecular diagnostic strategy for BS comprises direct (Sanger) sequencing or NGS-based multigene panel sequencing of *BLM*, as the majority of patients with BS/BS-like phenotypic features carry pathogenic variants in *BLM*. These NGS-based approaches, including WES analysis, should be considered as an important tool for the molecular diagnostics, especially as pathogenic variants in additional genes have been linked to a BS/BS-like phenotype.[13,14] We hypothesize that

patients in the BS registry[3] with no detected pathogenic variants in *BLM* could likely have causative variants in other *BLM*-related genes, which could be detected by NGS-based approaches.

In the current study, one novel frameshift variant in *BLM* (c.572_573delGA;p.Arg191Lysfs*4) was detected in two BS patients. The variant was classified as damaging by different in silico prediction tools including SIFT (https://sift.bii.a-star.edu.sg), MutationTaster (www.mutationtaster.org), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), and it is predicted to induce a frameshift resulting in a premature stop codon in the N-terminal region of the protein (Figure 1C). Parents of family 1 reported consanguinity while parents of family 2 claimed no relation although both were from the same village with less than 1000 inhabitants. Both families originated from the same country raising the possibility of a founder nature of this variant and suggesting a common ancestor haplotype. Furthermore, the c.581_582delTT variant that we identified in families 3 and 4 had been previously described in a single patient of Moroccan origin,[15] and this variant is present in the general human population with a minor allele frequency (MAF) of 8×10^{-6}, in line with an autosomal recessive pattern of inheritance (Table 1).

A distinct phenotypic characteristic of BS is the pronounced immunodeficiency, which is as important as any other life-threatening element of BS like cancer predisposition. We observed that all patients with pathogenic variants in *BLM* presented immunodeficiency resulting in recurrent infections, while patients with pathogenic variants in *RMI1* did not show this clinical feature, pointing to a phenotypic variety among the BS patients. The strongest phenotypic variance was observed between patients with LoF variants in different genes, namely *BLM* and *RMI1*. Both, *BLM* and *RMI1*, encode members of the BTRR complex; the major role within the complex may be assigned to BLM and TOP3A proteins due to their enzymatic activity on the DNA, although lack of RMI1 protein leads to defective cell proliferation as well.[17] Among the patients included in this study, individuals with pathogenic variants in *BLM* showed more severe BS phenotypic features compared to both individuals carrying the pathogenic variant in *RMI1*. This *RMI1* variant induces a frameshift predicted to lead to a truncated RMI1 protein. Currently, we cannot exclude expression and a residual function of this truncated RMI1 protein, which might result in a milder phenotype as observed in both patients carrying the *RMI1* variant. We conclude that pathogenic variants in the different members of the BTRR complex have a varying impact on the severity of the BS phenotype, especially when comparing *BLM*- and *RMI1*-associated phenotypes.

In summary, in eight patients presented with BS, we detected one novel frameshift variant in *BLM* in two patients from two families, two already known pathogenic variants in *BLM* in three families, and one truncating variant in *RMI1* in two individuals of a consanguineous family.[13] All variants were loss-of-function variants associated with the BS phenotype. Moreover, patients with pathogenic variants in the *BLM* gene showed more severe BS phenotypic characteristics in terms of photosensitivity, immunodeficiency, and infection rate than the patients carrying the *RMI1* variant. The immunodeficiency profile of BS is one of the most important characteristics of BS highlighting the importance to protect patients with BS against any kind of infection.

ACKNOWLEDGMENTS

The authors are grateful to all families for participating in this study and Karin Boss for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Research Group FOR 2800 "Chromosome Instability: Cross-talk of DNA replication stress and mitotic dysfunction", SP5 and SPZ (395736209) to B. Wollnik, by Germany's Excellence Strategy, Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC; EXC 2067/1-390729940) to B. Wollnik, and by German Center for Cardiovascular Research (DZHK, partner site Göttingen) to B. Wollnik.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

Written informed consent of all participants or their legal representatives was obtained prior to participation in the study. This study was performed according to the Declaration of Helsinki protocol and approved by the local institutional review board (University Medical Center Göttingen, Germany) under approval number 3/2/16.

DATA AVAILABILITY STATEMENT

The whole-exome sequencing raw data are not publicly available due to privacy or ethical restrictions.

ORCID

Ipek Ilgin Gönenc https://orcid.org/0000-0002-7583-0872
Carolina Martinez Grijalva https://orcid.org/0000-0002-0901-376X
Gökhan Yigit https://orcid.org/0000-0003-2777-0198
Bernd Wollnik https://orcid.org/0000-0003-2589-0364

REFERENCES

1. German J. Bloom syndrome, a mendelian prototype of somatic mutational disease. *Med (United States)*. 1993;72(6):393-406. doi:10.1097/00005792-199311000-00003
2. German J. Bloom's syndrome. I. Genetical and clinical observations in the first twenty-seven patients. *Am J Hum Genet*. 1969;21(2):196-227.
3. German J, Sanz MM, Ciocci S, Ye TZ, Ellis NA. Syndrome-causing mutations of the BLM gene in persons in the Bloom's Syndrome registry. *Hum Mutat*. 2007;28(8):743-753. doi:10.1002/humu.20501
4. Larsen NB, Hickson ID. *RecQ Helicases, Conserved Guardians of Genomic Integrity BT - DNA Helicases and DNA Motor Proteins*. Springer New York; 2013:161–184. doi:10.1007/978-1-4614-5037-5_8
5. Chu WK, Hickson ID. RecQ helicases: multifunctional genome caretakers. *Nat Rev Cancer*. 2009;9(9):644-654. doi:10.1038/nrc2682
6. Cunniff C, Bassetti JA, Ellis NA. Bloom's syndrome, clinical spectrum, molecular pathogenesis, and cancer predisposition. *Mol Syndromol*. 2017;8(1):4-23. doi:10.1159/000452082
7. Chaganti RSK, Schonberg S, German J. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc Natl Acad Sci U S A*. 1974;71(11):4508-4512. doi:10.1073/pnas.71.11.4508
8. Raynard S, Bussen W, Sung P. A double holliday junction dissolvasome comprising BLM, topoisomerase IIIα, and BLAP75. *J Biol Chem*. 2006;281(20):13861-13864. doi:10.1074/jbc.C600051200

9. Wu L, Bachrati CZ, Ou J, et al. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. *Proc Natl Acad Sci U S A*. 2006;103(11):4068-4073. doi:10.1073/pnas.0508295103
10. Singh TR, Ali AM, Busygina V, et al. BLAP18/RMI2, a novel OB-fold-containing protein, is an essential component of the Bloom helicase-double Holliday junction dissolvasome. *Genes Dev*. 2008;22(20):2856-2868. doi:10.1101/gad.1725108
11. Karow JK, Chakraverty RK, Hickson ID. The Bloom's syndrome gene product is a 3′-5′ DNA helicase. *J Biol Chem*. 1997;272(49):30611-30614. doi:10.1074/jbc.272.49.30611
12. Van Brabant AJ, Ye T, Sanz M, German JL, Ellis NA, Holloman WK. Binding and melting of D-loops by the Bloom syndrome helicase. *Biochemistry*. 2000;39(47):14617-14625. doi:10.1021/bi0018640
13. Martin CA, Sarlós K, Logan CV, et al. Mutations in TOP3A cause a bloom syndrome-like disorder. *Am J Hum Genet*. 2018;103(2):221-231. doi:10.1016/j.ajhg.2018.07.001
14. Hudson DF, Amor DJ, Boys A, et al. Loss of RMI2 increases genome instability and causes a Bloom-Like Syndrome. *PLoS Genet*. 2016;12(12):1-24. doi:10.1371/journal.pgen.1006483
15. Amor-Guéret M, Dubois-D'Enghien C, Laugé A, et al. Three new BLM gene mutations associated with Bloom syndrome. *Genet Test*. 2008;12(2):257-261. doi:10.1089/gte.2007.0119
16. Bloom D. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs, probably a syndrome entity. *AMA Am J Dis Child*. 1954;88(6):754-758. doi:10.1001/archpedi.1954.02050100756008
17. Yin J, Sobeck A, Xu C, et al. BLAP75, an essential component of Bloom's syndrome protein complexes that maintain genome integrity. *EMBO J*. 2005;24(7):1465-1476. doi:10.1038/sj.emboj.7600622

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Gönenc II, Elcioglu NH, Martinez Grijalva C, et al. Phenotypic spectrum of *BLM*- and *RMI1*-related Bloom syndrome. *Clinical Genetics*. 2022;101(5-6):559-564. doi:10.1111/cge.14125