

## REVIEW

# The Impact of Next Generation Sequencing in Diagnosis and Management of Rare Diseases: Bloom Syndrome

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## Abstract

Bloom syndrome is an exceptionally rare autosomal recessive disorder characterized by a considerable genomic instability due to the defective DNA damage repair machine. It is caused by biallelic pathogenic variants in the gene encoding for one of the five human RecQ helicases, RECQL3/BLM. The disorder manifests clinically as growth deficiency, skin anomalies, immunodeficiencies, insulin resistance, and a high predisposition to cancers. Less than 300 patients have been reported so far. In this paper, we report on the first Romanian patient of bi-ethnic origin, molecularly diagnosed with Bloom syndrome. As the most severe complications of the disorder are the malignancies, developing even in childhood, an early diagnosis is essential for further surveillance and therapeutic approach of Bloom patients.

**Keywords:** Bloom syndrome, chromosomal instability, helicase, DNA damage, cancer, sun sensitivity.

## Rezumat

Sindromul Bloom este o boală autozomal recesivă excepțional de rară, caracterizată printr-o considerabilă instabilitate genomică datorată deficienței mecanismului de reparare a ADN-ului. Aceasta este cauzată de variante patologice bialelice în gena care codifică una dintre cele cinci RecQ elicaze umane: RECQL3/BLM. Boala se manifestă clinic prin deficiență de creștere, leziuni cutanate, imunodeficiență, rezistență la insulina și o predispoziție marcată pentru cancer. Mai puțin de 300 de pacienți au fost raportați până în prezent. În acest articol, raportăm primul pacient roman cu origine bi-etnică care a fost diagnosticat molecular cu sindrom Bloom. Deoarece cea mai severă complicație a bolii este riscul de a dezvolta cancer chiar în copilărie, un diagnostic precoce este esențial pentru supravegherea și abordarea terapeutică ulterioară a pacienților Bloom.

**Cuvinte cheie:** sindromul Bloom, instabilitate cromozomială, elicază, afectare ADN, cancer, sensibilitate solară.

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Bloom syndrome (BSyn; MIM – Mendelian Inheritance in Man – 21090) is an exceptionally rare genetic condition characterized by considerable genomic instability due to a defective DNA damage repair machine. It is an autosomal recessive disorder, belonging to the group of chromosome instability syndromes, and it is caused by biallelic pathogenic variants in *RECQL3/BLM* (MIM 604610), a gene encoding for a helicase, which is a component of a protein complex that oversees the proper chromosomal recombination.<sup>1,2</sup> The others subunits of the complex: RecQ-mediated genome instability proteins 1 and 2, and topoisomerase III alpha, encoded by *RMI1*, *RMI2* and *TOP3A* genes, can also be involved in a similar phenotype called Bloom syndrome-like.<sup>3</sup> Genomic changes in any of these genes lead to a range of chromosomal aberrations including an increased frequency of sister-chromatin exchanges, loss of heterozygosity, chromosomal gaps, breaks, and translocations.<sup>4,5</sup> The clinical consequences consist in in utero and post-natal growth deficiency, short stature, microcephaly, skin anomalies, immunodeficiencies, insulin resistance, high predisposition to develop various malignancies in childhood or in early adulthood and sun sensitivity. The clinical feature that is detected at birth, or even before and is persistent throughout the patient's life, is growth restriction affecting all areas: head size, weight, and height. The short stature is proportionate and the level of growth hormone is normal. Feeding difficulties, consisting in decreased appetite and rejection of certain types of food, is often remarked in BSyn children and may contribute, along with gastro-esophageal reflux, vomiting and diarrhea, to growth failure.<sup>6</sup> Cutaneous findings may manifest as telangiectasia and erythema, distributed in a butterfly rash on the cheeks that may spread with sun exposure. Typically, the skin appears normal at birth or in early infancy and the rash becomes obvious during first or second year of life. However, the intensity and the extent of redness is variable among individuals, being slightest in some. Other skin lesions may include café-au-lait spots, hypopigmented macules and acanthosis nigricans.<sup>7</sup>

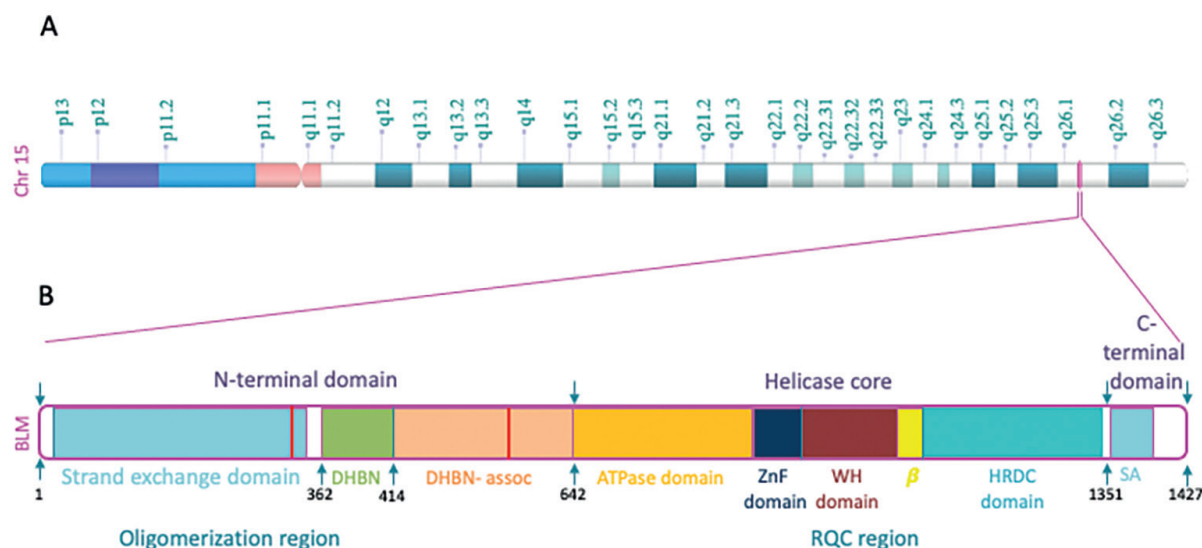
Besides insulin resistance and glucose intolerance resulting in an increased risk to develop type 2 diabetes, BSyn individuals may experience different endocrine comorbidities, such as: dyslipidemia, hypothyroidism and carbohydrate metabolism disturbances. Moreover, infertility has been often seen, especially in men, while in women, delayed puberty and early menopause have

been noted. Men with BSyn frequently display cryptorchidism and gonadal atrophy, primary hypogonadism, azoospermia or severe oligospermia.<sup>8</sup>

The most deleterious complications of the disorder are the multiple malignancies that constitute the main cause of mortality. Leukemia and lymphoma are the most common, followed by colorectal, breast and skin cancer. However, malignancies may interest almost any anatomic area including bones, tongue, oropharynx, esophagus, stomach, lung, kidney, and genitourinary system. Different types of cancer may occur in a single person at an early age, as opposed to other syndromes with predisposition to cancer.<sup>9</sup> The immune system is also affected in BSyn children, leading to a variety of recurrent infections. The quantification of serum immunoglobulins revealed, in most patient with BSyn, low levels of IgM and IgA, while IgG was decreased only in some individuals. The number of B cells and T cells are normal, but anomalies in cell proliferation or cell function have been reported in both T and B cell lines.

The disorder was first described in 1954 by David Bloom in three patients with primordial dwarfism, telangiectatic erythema resembling lupus erythematosus, and a bullous eruption on the lips as a result of sun exposure.<sup>10</sup> So far, less than 300 patients have been reported, and it seems prevalent in Ashkenazi Jewish population.

40 years later, the disease was associated with its causative gene: *BML* mapped to chromosome sub-band 15q26.<sup>11</sup> The gene has a size of 98 Mb (megabases) and is constituted of 22 exons. The encoded protein is one of the five human RecQ helicases that are involved in DNA unwinding during different stages of DNA replication, repair, transcription or recombination, turning the double-stranded DNA in two separate single strands.<sup>12</sup> It contains 1417 amino-acids distributed in: an N-terminal domain, a helicase core domain that includes an ATPase domain, a zinc finger domain, RQC domain and HRDC domain, and a C-terminal domain (Figure 1).<sup>3, 12-14</sup> The N-terminal domain is constituted of several subunits: the first one is the strand annealing/strand exchange (SA/SE) subdomain, involved in oligomerization and DNA loop formation by recruiting and interacting with other proteins.<sup>3</sup> The second less studied region of the N-terminal domain, known as Dimerization helical bundle in the N-terminal domain (DHBN), has been described, followed by the DHBN-associated region that is linked to the N-TD with helicase core domain.<sup>15</sup>



**Figure 1.** Figure 1: A\_ Ideogram of chromosome 15 showing the cytogenetic location of RECQL3/BLM gene (Genome Decoration Page: <https://www.ncbi.nlm.nih.gov/genome/tools/gdp>; B\_ Schematic representation of BLM protein and its domains: DHBN – Dimerization helical bundle in the N-terminal domain, DHBN-assoc – Dimerization helical bundle in the N-terminal domain associated region, ZnF – Zinc Fingers, WH – winged helix,  $\beta$  –  $\beta$ -hairpin domain, HRDC – helicase and RNase D C-terminal domain, SA – strand annealing domain; vertical red line mark the protein position of pathogenic variant identified in our patient.

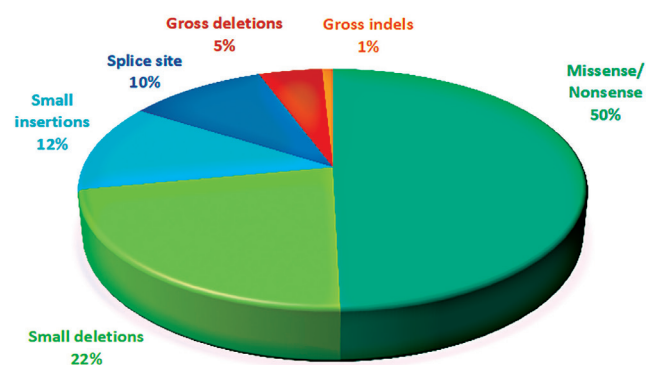
The ATP binding domain is the engine of the entire protein, containing two RecA like folds that use the ATP energy to translocate along DNA. The zinc finger domain binds the zinc ion that is required for ATPase and BLM helicase activities and stability.<sup>16</sup> The winged helix domain is also involved in helicase stability, being responsible for the insertion of the BLM complex between the two DNA strands, keeping them apart. The  $\beta$ -hairpin domain flips the nucleotides and dissolves the complementary strand of DNA, facilitating the complex sliding along the DNA molecule.

The helicase and RNase D C-terminal domain (HRDC) is not directly involved in helicase activity; however, given its specific affinity for ATP and its role in Holiday junction dissolution, it modulates the DNA binding.

The C-terminal domain contains an ssDNA annealing site that binds the DNA repair protein RAD51. RAD51 also binds to the N-terminal domain of the BLM protein, along replication protein A (RPA) and DNA topoisomerase 2-binding protein 1 (TopBP1), thus mediating the genome stability.

Disruption in any of these regions of the BLM protein leads to impairment of its function and consequently to disease. 126 deleterious pathogenic

variants have been reported in Human Gene Mutation Database (HGMD – <https://www.hgmd.cf.ac.uk/ac/index.php>) (Figure 2), the majority (62) being missense or nonsense mutation. Small deletions account for an important part of BSyn pathogenesis, as well, with 28 reported so far, followed by small insertions – 15, while splice site variants – 13 – represent a significant, albeit yet putative cause of BSyn. A smaller part of deleterious causative changes is represented by larger deletions



**Figure 2.** Types of pathogenic variants reported in Human Gene Mutation Database causing BSyn

/ indels - 6/1. Surprisingly, despite a small number of patients with BSyn identified so far, a high frequency was reported in persons of Ashkenazi Jewish ancestry, distinguishing some common mutation. The prevalence of carriers in Ashkenazi Jewish was estimated to one in 100. The variant c.2207\_2212delinsTAGATTC in exon 10 of *BLM*, also known as blm<sup>Ash</sup>, affecting the 736 position in Bloom helicase, p.Tyr736LeufsTer5, was identified as the prevalent pathogenic variant, followed by c.2407dupT, p.Trp803LeufsTer4. The second predominant allelic frequency was reported in Slavic population, counting for 0.2 to 0.8 in 100 individuals.<sup>17,18</sup>

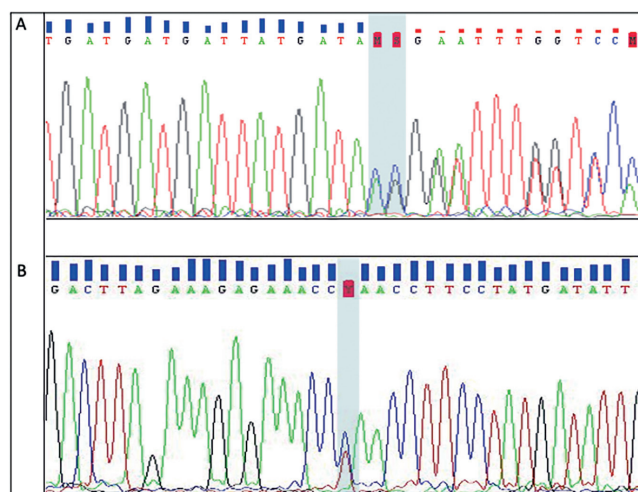
Here, we report on the first bi-ethnic, Slavic and Romanian, patient residing in Romania, harboring two pathogenic variants in *RECQL3/BLM*. A 2 years 3 months girl referred to our clinic for genetic evaluation. She is the first child of healthy, unrelated parents of Russian (the mother) and Romanian (the father) ethnicity. She has a clinical history of growth restriction and feeding difficulties, with a very decreased and highly selective appetite. She was born at term after an uncomplicated pregnancy. The physical measurement at birth revealed a weight at the lower percentile: 2,480 grams (3<sup>rd</sup> percentile), a head circumference below the standard: 31 centimeters (<1<sup>st</sup> percentile), while the length was within normal range: 49 centimeters (40<sup>th</sup> percentile). She had a good postnatal evolution and was normal in reaching the developmental milestones. Some minor cutaneous findings, two hyperpigmented spots, and one hypopigmented macula, raised the clinical suspicion of neurofibromatosis for which she was referred to genetic evaluation. Clinical genetic assessment has been done. At the time of presentation, she has a facial gestalt remarkable by narrow face, and a proportional “dwarf-appearance” with a small head and a paucity of subcutaneous fat. No facial rash, telangiectasias or sun sensitivity reactions have been noted. Her physical parameters were: weight 9 kg (<1<sup>st</sup> percentile), height 85 cm (20<sup>th</sup> percentile), occipitofrontal diameter (OFD) 42 cm (<1<sup>st</sup> percentile). Psychomotor development was normal. We decided to run a panel of genes including neurofibromatosis and growth restriction genes.

Next-generation sequencing was done on an Ion S5 platform (Thermo Fisher Scientific), using Ion AmpliSeq Exome RDY Kit, which allows the investigation of 99.9% of the coding sequences in the human genome. Raw data was processed using Torrent Suite v.5.12 and Ion Reporter v.5.16. The variants identified during the bioinformatic analysis were interpreted

based on data available in international databases: ClinVar, OMIM (Online Mendelian Inheritance in Man), dbSNP, UCSC (University of California, Santa Cruz) Genome Browser and others, using Congenica platform (Wellcome Trust). The analysis was done taking into account a minimum 20X coverage, in accordance with ACMG (American College of Medical Genetics and Genomics) guidelines. The identified variants were classified based on ACMG criteria.

Two compound heterozygous deleterious variants, one non-sense and one frameshift, in *RECQL3/BLM* gene were identified: the founder allelic variant in Slavic population: c.1642C>T, p.Gln548Ter (G548\*) and a new, unreported so far, variant: c.892dupA, p.Thr298AsnfsTer23 (Thr298fs). Since the mother is of Slavic origin, we can assume that G548\* was maternally inherited, while Thr298fs was paternally inherited, but the DNA of the parents was not available for testing. The c.1642C>T pathogenic variant in exon 7 affects the N-terminal domain of the encoded protein. The variant has been shown to result in a premature protein-translation termination at the 548 position that is predicted to produce a truncated protein. This variant is classified as pathogenic (class 5) according to ACMG/AMP standards, and has been reported before in patients with BSyn.<sup>18-20</sup> The second variant that results in a premature termination of the protein translation, c.892dupA, causes a frameshift mutation at position 298 in the oligomerization region of the BLM-protein-N-terminal domain and introduces a stop codon at position 23. The variant is classified as likely pathogenic (class 4) according to ACMG/AMP guidelines. This variant has not been reported in any dedicated database. Further *in vivo* or *in silico* studies are needed to predict the structural or functional consequences of the variant. However, the locus of stop codon insertion, upstream to other reported pathogenic variant, suggests a strong deleterious effect on protein. Both variants were validated by Sanger sequencing. (Figure 3)

Given that many patients diagnosed with BSyn do not display facial erythema, the clinical diagnosis is often delayed or difficult to predict.<sup>21</sup> However, similar to other rare diseases, an early diagnosis is valuable in anticipation and limitation of comorbidities.<sup>22</sup> Using sun protective lotion and avoiding prolonged sun exposure may be effective in the limitation of facial rash and may decrease the risk of skin cancer. Despite of growth improvement following growth hormone therapy, the



**Figure 3.** Sanger confirmation of pathogenic variants identified in our patient; A\_ frameshift variant c.892dupA; B\_ nonsense variant c.1642C>T

risk of early-onset-cancer-occurrence advocates for caution in its use or even its avoidance.<sup>23, 24</sup> Recurrent infections and immunodeficiency should be treated with gamma globulin injections, while type 2 diabetes, hypercholesterolemia or hypothyroidism should be addressed as in general population. A special attention should be accorded to BSyn individuals who develop cancers, as they have an increased risk of DNA-damage induced by chemotherapy or ionizing radiations. Reduced dosages of chemotherapeutic drugs and avoidance of some alkylating agents, especially busulfan, cyclophosphamide, or melphalan are required. Moreover, as much as possible, avoiding radiotherapy or using reduced doses of radiations and replacing of CT scans with other imagistic methods such as ultrasonography and MRI is recommended.<sup>6, 23</sup>

Periodic follow up is necessary for BSyn children. Abdominal ultrasounds should be done as soon as the diagnosis has been established, screening for Wilms tumors. Starting with age of 10, annual colonoscopy and serum dosage of glucose, lipids, thyroid hormone profile should be considered. Whole body MRI every two years beginning with 12 years is indicated.

As the most dramatic complications of BSyn are the malignancies, establishing the diagnosis may be life-saving for BSyn patients. Moreover, an adapted follow-up and therapeutic approach driven by chemo and radiation sensitivity of BSyn individuals, should be considered.

Thus, comprehensive molecular tests, in the absence of suggestive clinical clues may have a critical impact on diagnosis and further management of this patients.

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