

# Bloom Syndrome and Fanconi's Anemia: Rate and Ethnic Origin of Mutation Carriers in Israel

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## Abstract

**Background:** The Bloom syndrome gene, *BLM*, was mapped to 15q26.1 and its product was found to encode a RecQ DNA helicase. The Fanconi's anemia complementation group C gene was mapped to chromosome 9q22.3, but its product function is not sufficiently clear. Both are recessive disorders associated with an elevated predisposition to cancer due to genomic instability. A single predominant mutation of each disorder was reported in Ashkenazi Jews: 2281delATCT-GAinsTAGATTC for Bloom syndrome (BLM-ASH) and IV-S4+4AT for Fanconi's anemia complementation group C.

**Objectives:** To provide additional verification of the mutation rate of *BLM* and FACC in unselected Ashkenazi and non-Ashkenazi populations analyzed at the Sheba Medical Center, and to trace the origin of each mutation.

**Methods:** We used polymerase chain reaction to identify mutations of the relevant genomic fragments, restriction analysis and gel electrophoresis. We then applied the Pronto™ kit to verify the results in 244 samples and there was an excellent match.

**Results:** A heterozygote frequency of 1:111 for BLM-ASH and 1:92 for FACC was detected in more than 4,000 participants, none of whom reported a family history of the disorders. The Pronto™ kit confirmed all heterozygotes. Neither of the mutations was detected in 950 anonymous non-Ashkenazi Jews. The distribution pattern of parental origin differed significantly between the two carrier groups, as well as between each one and the general population.

**Conclusions:** These findings as well as the absence of the mutations in non-Ashkenazi Jews suggest that: a) the mutations originated in the Israelite population that was exiled from Palestine by the Roman Empire in 70 AD and settled in Europe (Ashkenazi), in contrast to those who remained; and b) the difference in origin distribution of the BS and FACC mutations can be explained by either a secondary migration of a subgroup with a subsequent genetic drift, or a separate geographic region of introduction for each mutation.

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Bloom syndrome and Fanconi's anemia complementation group C are recessive disorders associated with excessive genomic instability and an increased risk for malignancy [1-3]. The major clinical manifestations of BS are short stature, sensitivity to the sun, erythema, and immunodeficiency. The somatic cells of patients with BS are hypermutable with an excessive number of chromatid gaps, breaks and sister-chromatid exchange [1]. FACC is characterized by multiple congenital abnormalities and bone marrow failure [2-4], and these patients' cells show hypersensitivity to DNA cross-linking agents [2,4].

The BS gene *BLM* was cloned in 1995, mapped to 15q26.1 and its product encodes a RecQ DNA helicase [5]. FACC gene (*FAC*) was mapped to chromosome 9q22.3 [6], but the cellular function of its product is still unclear. There are indications that it forms a nuclear complex with Fanconi's anemia group A protein [7] and interacts with a cyclin-dependent kinase [8].

Bloom syndrome is far more common among Ashkenazi Jews, especially of Polish origin, than any other ethnic group [9,10]. Results of population studies point strongly to a founder effect with a single predominant mutation, 2281delATCTGAinsTAGATTC, which can be identified in all Jewish patients [5]. Different laboratories have estimated the allele carrier frequency to be between 1:101 and 1:110 [11,12]. Similarly, a single mutation within the FACC gene, IVS4+4AT, was found in Ashkenazi Jewish patients and its frequency was estimated to be 1:89 to 1:98 [13-15].

The present study seeks to provide further verification of the rate of *BLM* and FACC predominant Jewish mutations in an unselected large Ashkenazi and non-Ashkenazi Jewish population in Israel (n=4,979) and attempts to determine the geographic point of outburst of each of the founder alleles.

## Materials and Methods

### Patients

Genetic testing to detect BS and FACC carriers was part of the screening service conducted at the Sheba Medical Center between December 1997 and April 2000. Each participant completed a routine questionnaire on family history and the ancestry of his or her four grandparents. These screening tests were not recommended to non-Ashkenazi individuals (i.e., those with two non-Ashkenazi parents). There was no reported consanguinity or positive family history of BS or FACC among

FACC = Fanconi anemia complementation group C

BS = Bloom syndrome

the carrier population. The vast majority of participants (above 95%) were of Ashkenazi origin (i.e., with four Ashkenazi grandparents), while the rest had one parent of Ashkenazi ancestry. In addition, 950 non-Ashkenazi individuals (originating in North Africa, Persia, India or Yemen) were included in the study.

### Mutation detection

The Puregene Kit (Gentra, Inc, USA) was used to isolate DNA from blood samples according to the manufacturer's instructions. The BS mutation (2281-6bpdel/7bpins) introduces a novel BstNI restriction enzyme site, resulting in two extra bands of 247 and 90 base pairs in addition to the normal 337 bp band [11]. FACC mutation (IVS4) abolishes a ScaI restriction site, resulting in a single band of 130 bp instead of the two wild-type shorter bands of 107 and 103 [16]. The overnight digested PCR products (MJ Research, Inc., USA) were separated on 2% and 4% agarose gel (Seakem, MBA, USA) for BS and FACC, respectively, and visualized with an ultraviolet lamp following ethidium bromide staining (Bio Imaging System 202D, Israel). Known heterozygote and homozygote normal samples were included in every run. Each heterozygote sample was tested twice for confirmation. We re-analyzed 200 normal samples and 51 mutation carriers for Bloom-Fanconi mutations with the Pronto™ kit (Savyon Diagnostics Ltd, Israel) for the purpose of comparison and additional confirmation; the procedure was carried out according to the manufacturer's instructions.

### Data analysis

The origins of the four grandparents of each patient were included in the birthplace registry and the allele population pool. The distribution of allele origin (according to the questionnaires) was compared: a) between carriers of the two disease genes, and b) between carriers and non-carrier alleles. The significance of differences between groups was analyzed by the chi-square test.

### Results

A total of 4,001 individuals were routinely tested for the Ashkenazi mutation of Bloom syndrome (2281 del/ins mutation) and 4,029 individuals for Fanconi's anemia (IVS4T). All tested individuals were of Ashkenazi origin. Frequencies of carriers were found to be 1:111 (0.009) and 1:92 (0.0109) for BS and FACC respectively [Table 1]. No mutation carriers were detected among 950 anonymously tested non-Ashkenazi Jews (from North Africa and the Middle East). Twenty-four of the BS carriers and 27 of the FACC carriers (including one double carrier) were tested also by the Pronto™ kit to confirm the diagnoses and the results matched perfectly. In addition, 192 randomly chosen non-carriers were re-tested by the Pronto™ kit. The results disclosed a FACC carrier in one sample, but subsequent tests using both methods (correlation coefficient above 0.98) yielded normal findings.

Table 2 details the distribution of 144 ancestor alleles of 36

bp = base pairs  
PCR = polymerase chain reaction

**Table 1.** Frequency of carriers among individuals of Ashkenazi origin

Disease	Total no. of tests	No. of carriers	Carrier frequency*	Gene Frequency
Bloom	4,001	36	0.009 (1:111)	0.0045
Fanconi	4,029	44	0.0109 (1:92)	0.0054

\* Calculated confidence intervals (95%) for the carrier's frequency: Bloom syndrome 0.0061–0.0012, Fanconi anemia 0.0078–0.014.

**Table 2.** Ethnic background of the population's allele pool

Country of origin	Fanconi heterozygotes	Bloom Heterozygotes	Non-carrier controls
1. Poland	65 (36.9)	64 (44.4)	862 (37.6)
2. Russia	30 (17)	39 (27.1)	545 (23.8)
3. Rumania	28 (15.9)	14 (9.7)	358 (15.6)
4. Germany	15 (8.5)	5 (3.5)	137 (6.0)
5. Czech Republic	12 (6.8)	2 (1.4)	71 (3.1)
6. Hungary	9 (5.1)	1 (0.7)	73 (3.2)
7. Austria	2 (1.1)	3 (2.1)	41 (1.8)
8. Ashkenazi*	10 (5.7)	10 (6.9)	132 (5.8)
9. Non-Ashkenazi	3 (1.7)	3 (2.1)	53 (1.9)
10. Israel**	2 (1.1)	3 (2.1)	15 (0.65)
<b>Total allele no.</b>	<b>176</b>	<b>144</b>	<b>2288</b>

Numbers in parentheses represent the percent of the group's total.

\* Country of origin was not specified, but the respondents were certain about the Ashkenazi ethnicity.

\*\* Born in Israel several generations ago, but are not sure about the origin.

BS carriers, 176 grandparent alleles of 44 FACC carriers, and 2,288 control alleles (572 non-carriers), sorted according to their origins. Individuals with no specified country of origin and non-Ashkenazi parents were excluded from the analysis (groups 8–10, Table 2). The distribution of the three groups (Fanconi's anemia carriers, BS carriers and non-carriers) among the seven countries of origin (origins 1–7 in Table 2) differed significantly from each other [Table 3]. The difference between the ethnic distributions of the two carrier groups was highly significant, while the difference between each of them and the control population was less substantial (not statistically significant in the case of BS heterozygotes) [Table 3]. In order to exclude biased results due to the very small size of the populations, we combined groups 4–7 [Table 2] into one and re-analyzed the distribution according to four main classes. The distribution pattern among the four origin groups differed significantly between the two carrier groups and between each one compared to the general population [Table 3, column 3].

There was a small excess (with borderline significance,  $P=0.05$ ) in the proportion of Polish-descent alleles among BS carriers as compared to the general population group, whereas among the FACC carriers the contribution of the "Polish" allele pool was similar to that of the general population.

### Discussion

The observed rate of BS and FACC heterozygotes (1:111 and 1:92, respectively) among the Ashkenazi Jewish populations is in agreement with previously reported values [9–15] for this ethnic group. The current results are based on approximately 4,000

**Table 3.** Chi-square analysis of the ethnic distribution differences

Analysis	7 origin groups*	4 origin groups**
FACC vs. BS vs. Control	$P = 0.0099$	$P = 0.0027$
FACC vs. BS	$P = 0.0017$	$P = 0.0006$
FACC vs. Control	$P = 0.0316$	$P = 0.0229$
BS vs. Control	$P = 0.0840^{***}$	$P = 0.0226$

\* Analysis of the allele distribution among countries of origin 1–7 (detailed in Table 2). \*\* Countries of origin 4–7 were combined into a single origin class. The analysis was repeated using four regions of origin: 1–3 (according to Table 2) and the combined group. \*\*\* Not significant.

individuals, the vast majority of whom have “full” Ashkenazi ancestry [Table 2]. To the best of our knowledge this is the largest study reported. The increased frequency of a single BS mutation among all Ashkenazi Jews, coupled with linkage disequilibrium data [17], provided strong evidence for a founder ancestor [9–11]. A recent study [18] reported the diagnosis of this “Jewish” mutation in five non-Jewish families who dwelt for generations in southern California, El Salvador and Mexico. Haplotype analysis in these families was consistent with a possible Ashkenazi Jewish ancestor who probably introduced the mutation to a common Spanish founder.

The IVS4T mutation of FACC was found in about 84% of the Ashkenazi-Jewish affected alleles. A founder effect, followed by genetic drift, has been suggested as responsible for the increased rate of this mutation among Ashkenazi Jews. Recently, this mutation was found in eight unrelated Japanese patients – the first time in a non-Jewish population [19]. Unlike the Jewish homozygous patients, in the Japanese patients this mutation is not associated with a severe phenotype. There was one double mutant, approximately as expected (up to one) according to the population’s carrier frequency, assuming that there is no association between the two mutations.

An excess of BS heterozygotes was reported among Ashkenazi Jews of Polish origin [9], however no particular candidate region was proposed as the origin for the FACC mutation. According to the present results, countries other than Poland and Russia (Rumania, Czech Republic, Hungary and Germany) contributed significantly to the allele pool of FACC carriers. This different distribution among countries of origin might be the consequence of a secondary migration of subgroups followed by genetic drifts, or may be due to the separate introduction (or involvement) of the mutations in two different regions. The lack of IVS4+4 and 2281-6bpdel/7bpins in non-Ashkenazi populations [9,14] implies the possibility that the mutations originated in the population that was separated from the Israelite population and exiled from Palestine by the Roman Empire in 70 AD.

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