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Linkage Disequilibrium between the *FES*, *D15S127*, and *BLM* Loci in Ashkenazi Jews with Bloom Syndrome

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Summary

Bloom syndrome (BS) is more common in the Ashkenazi Jewish than in any other population. Approximately 1 in 110 Ashkenazi Jews carries *blm*, the BS mutation. The locus mutated in BS, *BLM*, maps to chromosome subband 15q26.1, tightly linked to the proto-oncogene *FES*. We have investigated the basis for the increased frequency of *blm* in the Ashkenazim by genotyping polymorphic microsatellite loci tightly linked to *BLM* in affected and unaffected individuals from Ashkenazi Jewish and non-Ashkenazi populations. A striking association of the C3 allele at *FES* with *blm* ($\Delta = .422$; $p = 5.52 \times 10^{-7}$) and of the 145-bp and 147-bp alleles at *D15S127* with *blm* ($\Delta = .392$ and $\Delta = .483$, respectively; $p = 2.8 \times 10^{-5}$ and $p = 5.4 \times 10^{-7}$, respectively) was detected in Ashkenazi Jews with BS. This linkage disequilibrium constitutes strong support for a founder-effect hypothesis: the chromosome in the hypothetical founder who carried *blm* also carried the C3 allele at *FES* and either the 145-bp or the 147-bp allele at *D15S127*.

Introduction

Bloom syndrome (BS) is a rare autosomal recessive trait characterized clinically by growth deficiency, a sun-sensitive telangiectatic erythema of the face, immunodeficiency, and male infertility (German 1993). Somatic cells from persons with BS are characterized by a striking genomic instability. They display an increased frequency of chromosome abnormalities (breaks, gaps, and rearrangements) and inter- and intramolecular chromosome exchanges, including sister-chromatid exchanges (Ray and German 1983). One major consequence of this genomic instability is an increased risk of neoplasms of the types and sites that arise in the general population (German 1993).

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Hybridization of a cell from a normal individual with a BS cell results in correction of the increased sister-chromatid exchange that is uniquely characteristic of BS cells (Bryant et al. 1979). Similar studies in which the fusion partners were cells from persons with BS from several different ethnic groups (western Europeans, Ashkenazi Jews, and Japanese) indicated that mutation at a single locus is responsible for BS (Yoshida 1981; Weksberg et al. 1988). That locus was shown to be on chromosome 15 by a microcell chromosome-mediated gene-transfection technique (McDaniel and Schultz 1992).

In order to map the locus mutated in BS to a specific chromosome segment, a linkage study was conducted employing *homozygosity mapping*—an approach to gene mapping whose value has been reemphasized recently (Lander and Botstein 1987). In 20 of 21 families in which the parents are related (referred to hereafter as *consanguineous families*), a polymorphic microsatellite in the proto-oncogene *FES* was homozygous in the persons with BS, whereas the frequency of homozygosity in the general population is 33% (German et al., 1994). This excessive degree of homozygosity of *FES* in affected persons from consanguineous families indicated that *BLM*, our term for the BS locus, is tightly linked to *FES*, a gene that is situated in 15q26.1 (Jhanwar et al. 1984; Mathew et al. 1993).

BS is more common in Ashkenazi Jews than in any other population that has been studied (German et al. 1977). In the Ashkenazim, ~1 in 110 individuals carries *blm*, our term for the BS mutation(s); elsewhere *blm* is extremely rare. In 34 of the 137 families in the Bloom's Syndrome Registry (BSR), both parents are Ashkenazi Jewish. Of the 34 Ashkenazi Jewish families in the BSR, only 2 are consanguineous, whereas 32 of the 95 non-Ashkenazi families are.

The increased frequency of *blm* in Ashkenazi Jews might be explained by the founder-effect hypothesis (Mayr 1942). The Ashkenazim are thought to be derived from Jews of Palestinian/Mediterranean origin who had migrated into the Rhineland in the 9th century and who later migrated in the 13th and subsequent centuries into the then sparsely populated areas of present-day Poland, Lithuania, Belarus, and Ukraine (Ankori 1979). According to the founder-effect hypothesis, one of the early immigrants to eastern Europe by chance carried a *blm* allele, and by

genetic drift it became more frequent in the Jews inhabiting eastern Europe (Wright 1921).

As stated above, *FES* is tightly linked to *BLM*. Might not an association in the postulated founder of, say, one particular allele of *FES* and *blm* be observed in present-day Ashkenazi Jews with BS? Here we report findings that lend strong support to founder effect as the basis today for the relatively high incidence of BS in Ashkenazi Jews.

Subjects and Methods

Sources of Population Samples

DNA samples were collected from four populations: (1) persons with BS both of whose parents are Ashkenazi Jewish— "BS/Ashkenazi," (2) persons who do not have BS both of whose parents are Ashkenazi Jewish—"non-BS/Ashkenazi," (3) persons with BS neither of whose parents are Ashkenazi Jewish—"BS/non-Ashkenazi," and (4) persons who do not have BS whose parents are not Ashkenazi Jewish—"non-BS/non-Ashkenazi." Population 3 was analyzed in two ways by including or excluding affected individuals whose parents are consanguineous; the results were the same whether or not such individuals were excluded so that all were kept in a single group.

Samples for the BS/Ashkenazi and BS/non-Ashkenazi populations were obtained from the BSR (German and Passarge 1989): pedigrees, clinical data, and information pertaining to geographic and ethnic origins of most of the persons who were diagnosed with BS between 1954 (when the disorder was first reported) and 1991 have been accumulated in the BSR files. Samples of blood and/or skin were collected during this period. Whole blood, isolated lymphocytes, and/or isolated granulocytes were stored in liquid nitrogen. When possible, lymphoblastoid or fibroblast cell lines were developed. In this study, ~40% (55 of 137) of the families in the BSR were sampled. Several additional samples were obtained from individuals with BS who were ascertained since January 1, 1991, when accession of families to the BSR was discontinued. The geographic and ethnic origins of the grandparents of most of the individuals with BS examined in this study are shown in table 1.

For the non-BS/Ashkenazi group, samples of purified blood lymphocytes that had been cryopreserved from 50 individuals were provided by Pablo Rubinstein and Cladd Stevens at the New York Blood Center. These samples were originally collected from Ashkenazi Jewish persons dwelling in New York City, as part of an unrelated study. The donors are not known to be related to any person with BS, and they each have four Ashkenazi Jewish grandparents each of whom was born in roughly the same part of Europe as the ancestors of the Ashkenazi Jewish BS families. The non-BS/non-Ashkenazi group was composed of DNA samples from unrelated CEPH grandparents from 40 CEPH families.

DNA Preparation

Fresh or cryopreserved cells in whole blood or from granulocyte fractions were disrupted in whole-blood lysis buffer (0.32 M sucrose, 10 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, and 1% Triton X-100), and the nuclei were pelleted by centrifugation at 1,000 *g* for 15 min at 4°C. PBS-washed cells from cultured lymphoblastoid cell lines, fibroblast cell lines, isolated lymphocytes from whole blood, or pelleted nuclei from whole blood or granulocyte fractions were lysed in 0.5 M NaCl, 0.2% SDS, and 10 mM EDTA. Proteinase K was added to 0.1–0.2 mg/ml, and digestion was carried out at 50°C overnight. The DNA was extracted once in phenol/chloroform, then once in chloroform, and finally precipitated by the addition of $2^1/_2$ vol of ethanol. DNA isolated from blood samples that had been collected in heparin was resistant to restriction-endonuclease digestion; to restore the capacity to be digested, such samples were dialyzed against 10,000 vol of TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The DNA isolated from non-BS/Ashkenazi was extracted from purified, previously cryopreserved (with DMSO) blood lymphocytes, by a salting-out technique (Miller et al. 1988).

Genotyping of Polymorphic Microsatellites

Genotypes at the following loci were determined: *FES, D15S111, D15S112, D15S116, D15S127,* and *D15S130.* Alleles of these polymorphic microsatellites were determined by PCR (German et al., 1994). Information on oligonucleotide primers, expected allele sizes, and distribution of allele frequencies was obtained from the Genome Database at the Welch Medical Library, Johns Hopkins University.

Statistical Analysis of Linkage Disequilibrium

Estimates of allelic association were calculated using the standardized disequilibrium coefficient Δ: $\Delta = (p_{AB} - p_A p_B)/\sqrt{p_A p_a p_B p_b}$, where p_{AB} is the frequency of alleles A and B together, p_A is the frequency of the A allele, p_B is the frequency of the B allele, and p_a and p_b represent the total frequencies of all other alleles at loci A and B, respectively. The numerator of Δ, representing the classical definition of linkage disequilibrium, is sensitive to the allele frequencies and so is a less informative statistic than Δ, which is independent of allele frequencies. Significance of Δ was measured by Fisher's exact probability.

The frequency of the *blm* allele in the Ashkenazi Jewish population was calculated from the carrier frequency (.0091) taken from German et al. (1977), under the assumption of Hardy-Weinberg equilibrium. Following the arguments of Lehesjoki et al. 1993, we estimated the proportion of all mutation-bearing chromosomes that have new mutations (i.e., are not descended from the founder allele) to be $\gamma = \mu t/q$, where μ is the mutation rate from normal to disease allele (assumed to be 5×10^{-6}/cell/gen-

Table 1

Geographic Origins of Grandparents of Persons with BS Who Were Genotyped

BSR Code[a]	Paternal Grandfather	Paternal Grandmother	Maternal Grandfather	Maternal Grandmother
Ashkenazi Jewish:				
53(StAs)	Poland		Russia	Austria/Poland
3(HoCo)	Poland-Russia	United States	Austria	Austria
57(AmEl)	Poland	Ukraine	Poland	Poland
42(RaFr)	Ukraine			
56(JoGr)	Poland	Poland	Ukraine	United States
(AkGro)	Russia	Russia		Poland
120(MiHal)	Poland	Hungary		
106(JaHe)	Poland-Russia	Poland-Russia	Poland-Russia	Poland-Russia
32(MiKo)			Poland-Russia	Poland-Russia
142(MaMat)	Ukraine	Ukraine	Poland	Poland
121(NiRos)	Ukraine	Poland	Germany	Sephardim
15(MaRo)	Poland	Ukraine	Ukraine	Ukraine
44(AbRu)	Russia	Russia		United States
27(LySe)	Germany	Germany	Poland-Russia	Poland-Russia
9(EmSh)	Poland	Russia	Russia	Russia
14(LeSi)	Poland	Poland	Poland	Lithuania
47(ArSmi)	Ukraine	Ukraine	Ukraine	United States
79(MeDer)[b]	Poland	Russia[c]	Russia[c]	United States
Non-Ashkenazi:				
Nonconsanguineous:				
(ErBor)	France	France	Ireland	Ireland
59(FrFit)	United States	French-Canada	Germany	
108(IsGre)	United States	United States	United States	United States
(KeHeg)	Germany/Holland	Bohemia	Sweden	Sweden
111(JaKir)				
139(ViKre)	Germany	Bohemia/England	Germany/Sweden	Germany/Sweden
(RaLe)				
145(WeNo)	Sweden	Ireland/Holland		
80(ErPal)	Italy	Italy	Italy	Italy
65(AnPa)	Holland	Holland	Holland	Holland
40(DoRoe)	Bessarabia	Hungary	United States	United States
67(SuSc)	Holland	Holland	Holland	Holland
112(NaSch)			United States	United States
20(ViShr)	United States	United States	United States	United States
11(IaTh)	Scotland	England	England	England
130(ChVa)	United States	United States	United States	United States
105(ShWo)	United States	United States	United States	Germany
Consanguineous:				
74(OmAy)	Turkey	Turkey	Turkey	Turkey
92(VaBia)	Italy[c]	United States	Italy	Italy[c]
60(AnDv)	United States[c]	United States	United States	United States[c]
113(DaDem)	Italy	Italy[c]	Italy[c]	Italy
52(PaDu)	Germany	Germany[c]	Germany	Germany[c]
81(MaGrou)	French Canada[c]	French Canada	French Canada	French Canada[c]
22(ElHa)	United States[c]	United States	United States	United States[c]
122(RoHer)	United States[c]	United States	United States[c]	United States
61(DoHop)	United States	United States[c]	United States	United States[c]
140(DrKas)	Sephardim[c]	Sephardim	Sephardim	Sephardim[c]
30(MaKa)	Germany[c]	Germany	Germany[c]	Germany
110(MaKur)	Japan[c]	Japan	Japan[c]	Japan
127(TaLu)	United States[c]	Germany	United States[c]	United States
51(KeMc)	United States	United States[c]	United States[c]	United States
5(JaOa)	Germany[c]			Germany[c]
96(HiOk)	Japan	Japan[c]	Japan	Japan[c]
21(RaRe)	United States[c]	United States	United States	United States[c]
149(SeSaf)	Turkey[c]	Turkey	Turkey[c]	Turkey
17(ChSm)	United States[c]	United States	United States	United States[c]
7(RoTa)	Italy[c]	Italy	Italy[c]	Italy

NOTE.—In cases where a grandparent was born in the United States, information on the great-grandparents has been used, provided that their origins are more specific. "United States" usually means that the person was of Anglo-Saxon origin, with the notable exceptions of the grandparents of 130(ChVa), who were Spanish Catholics, and 108(IsGre), 60(AnDav) and 122(RoHer), who have African origins. "Poland-Russia" has been used when the respondent did not specifically name the town or country of origin but did indicate Ashkenazi ancestry. "Sephardim" has been used to indicate Jews whose ancestors migrated from Spain in the 15th century. Blanks indicate that no information was available.

[a] Accession name of the Bloom's Syndrome Registry.

[b] Person was born to consanguineous parents.

[c] Person was in the consanguinity loop.

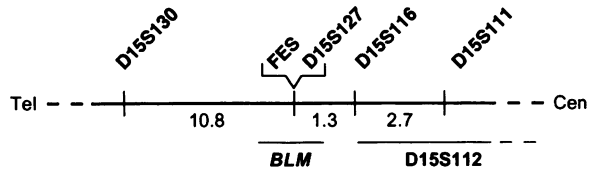

Figure 1 Map position of *BLM* relative to tightly linked markers. The relative positions of (*D15S111, D15S112*), *D15S116*, (*D15S127, FES*), and *D15S130* have been reported (Beckmann et al. 1993). *FES* and *D15S127* are as yet unseparated by recombinational events. The order of the loci on a thinner line offset from the thicker line, with respect to flanking loci, is unknown. Below the map, the map distances (in cM) are shown for those loci for which published values exist. By homozygosity mapping, *BLM*, *FES*, and *D15S127* are situated in an interval of ~1 cM (German et al. 1994).

eration), t is the number of generations since the origin of the population or the introduction of the *blm* allele (here assumed to be the number of generations since Jews migrated into eastern Europe, or roughly 30 generations), and q is the frequency of the *blm* allele. Similarly, we estimated the recombination fraction, θ, between the *BLM* and *FES* loci from the equation $(1-\theta)^t = \alpha\pi/(1-\mu tq^{-1})$, where $\alpha = 1-\gamma$ and π is the proportion of current mutation-bearing chromosomes, descended from the founder, that have not undergone a recombinational event (for details of these formulas, see the Appendix).

Results

Homozygosity Mapping Using Markers Tightly Linked to FES

We have confirmed and extended the data used to identify linkage between *FES* and *BLM* (German et al. 1994), by determining the genotypes of affected persons from consanguineous families, at the following loci tightly linked to *FES: D15S111, D15S112, D15S116, D15S127,* and *D15S130*. The most likely relative order and map distances of these loci is shown in figure 1 (Beckmann et al. 1993). As stated above, in 20 of 21 consanguineous families the affected persons were homozygous at *FES* (see table 2). Polymorphic markers that flank *FES*—i.e., *D15S111, D15S112, D15S116, D15S127,* and *D15S130*—also were homozygous in a majority of the affected persons (table 2). To calculate the expected number of homozygotes and heterozygotes, the published heterozygosity values at each locus derived from unrelated parents or grandparents in CEPH families were used. The proportion of affected persons homozygous at these loci was significantly greater than that expected (table 2). The three most tightly linked loci—*FES, D15S127,* and *D15S116*—were chosen for genotyping in the Ashkenazi Jewish population in our search for linkage disequilibrium.

Linkage Disequilibrium between BLM *and the* FES, D15S127, *and* D15S116 *Loci*

The *FES* polymorphism, a tetranucleotide repeat in intron V, has six alleles C1–C6 (Polymeropoulos et al. 1991). The *D15S127* polymorphism has >20 alleles, and the *D15S116* polymorphism has ≥15 alleles (Beckmann et al. 1993; also see tables 3–5). The genotypes of the *FES, D15S127,* and *D15S116* polymorphisms of the DNA samples collected for this study were determined by PCR, and the allele frequencies were then calculated for affected and unaffected persons from both Ashkenazi and non-Ashkenazi populations (tables 3–5). The frequencies of the *FES* and *D15S127* alleles in the BS/Ashkenazi chromosomes were significantly different from those in the non-BS/Ashkenazi. In particular, the *FES* C3 allele was represented at a frequency of .88 in the BS/Ashkenazi chromosomes but at a frequency of only .39 in the non-BS/Ashkenazi. Association between the *blm* and *FES* C3 alleles as measured by Δ is .422 ($p = 5.52 \times 10^{-7}$). At the *D15S127* locus, two alleles were significantly different between the two samples: the *D15S127* 145-bp and 147-bp alleles had frequencies of .45 and .39, respectively, in the BS/Ashkenazi chromosomes, versus only .10 and .03 in the non-BS/Ashkenazi. This results in Δ values for the *D15S127* 145-bp and 147-bp alleles that are .392 ($p = 2.8 \times 10^{-5}$) and .483 ($p = 5.4 \times 10^{-7}$), respectively. At the *D15S116* locus, the 7 allele is more frequently associated with *blm* than are other alleles, resulting in a Δ value of .173 ($p = .045$). Just as the linkage between *BLM* and *D15S116* is weaker than that between *BLM* and *FES* or *D15S127* (table 2), so is the linkage disequilibrium.

Significant differences in *FES* and *D15S116* allele frequencies between non-BS/Ashkenazi, BS/non-Ashkenazi, and non-BS/non-Ashkenazi chromosomes were not observed (see tables 3 and 5). On the other hand, the frequencies of the *D15S127* 132-bp and 138-bp alleles are increased, and those of the 114-bp, 135-bp, and 141-bp alleles are decreased, in BS/non-Ashkenazi chromosomes compared with those in non-BS/non-Ashkenazi chromosomes (see table 4). These results show that strong linkage disequilibrium exists between *FES, D15S127,* and *BLM* in Ashkenazi Jews who are homozygous for *blm*.

Discussion

The linkage disequilibrium between *FES* and *BLM* in the BS/Ashkenazi population reported in the present study constitutes strong support for founder effect as the basis of the increased frequency of BS in Ashkenazi Jews. This explanation can be articulated in the following way: (1) A single individual who by chance carried a mutant allele of *BLM*, arbitrarily named blm^{Ash} here, migrated into sparsely populated eastern Europe sometime during the 13th or 14th centuries. (2) Genetic isolation there of Jews led to a reduction in the effective population size. (3) The blm^{Ash} gene thereafter increased in frequency by genetic drift (Wright 1921). In the hypothetical founder, the chromosome that carried blm^{Ash} also carried the *FES* C3 allele, either the *D15S127* 145-bp or 147-bp alleles, and very likely the *D15S116* 7 allele.

Table 2

χ^2 Analysis of the Frequency of Homozygotes and Heterozygotes in Persons with BS Whose Parents Are Cousins: Polymorphic Loci on Chromosome 15, Tightly Linked to *FES*

Locus	Heterozygosity in the General Population	Heterozygotes in BS		Homozygotes in BS		χ^2
		Observed	Expected	Observed	Expected	
D15S111	.74	5	14.8	15	5.2	24.96*
D15S112	.68	4	13.6	16	6.4	21.18*
D15S116	.86	7	16.0	12	3.0	32.06*
D15S127	.87	4	18.3	17	2.7	86.91*
FES	.67	1	14.1	20	6.9	37.04*
D15S130	.71	10	14.2	10	5.8	4.28**

* $P < .001$.
** $P < .05$.

While the analyses of genotypic data at *FES* strongly support the founder-effect hypothesis, those of genotypic data at *D15S127* are less strong. Several explanations could account for the association between blm^{Ash} and two alleles, rather than a single allele, of *D15S127:* (1) Because *D15S127* and *BLM* are tightly linked and the hypothetical founder lived ~30 generations ago (under the assumption that there are 4 generations every 100 years and that the founder's migration date into eastern Europe was 1250 A.D.), the process of recombination could have dissociated the original combination of *D15S127* 145-bp or 147-bp alleles and blm^{Ash} that existed on one of the founder's chromosome 15s. A recombinational event between *D15S127* and *BLM* early in the descent of the blm^{Ash} gene from the founder to present-day Ashkenazi Jews could have established the two major haplotypes on which the blm^{Ash} mutation now is found. Similarly, the other alleles of *FES* and *D15S127* that today are present on blm^{Ash} chromosomes could be derived from recombinational events that occurred in later generations (tables 3 and 4). (2) Mutations that vary the number of repeats in the *D15S127* polymorphism would have the same effect as recombination. Supporting this second possible explanation, we have detected one affected person who inherited an allele of *D15S127* that was not found in either of his parents (data not shown). Also in support of the mutation hypothesis, we have discovered that *FES* and *D15S127* can be carried on the same P1 clone (see Pierce et al. 1992), indicating that these loci lie <85 kb apart (data not shown); it is unlikely that recombination would have occurred in such a small physical interval in an early Ashkenazi ancestor who carried blm^{Ash}. (3) Finally, and perhaps least likely, two Ashkenazi Jewish founders might have introduced different blm^{Ash} mutations, or an additional founder from a different population might have entered the Ashkenazi Jewish population. These hypotheses can be distinguished definitively after the *BLM* gene is cloned and the *blm* mutations are characterized. We will be able then to identify blm^{Ash} mutations in BS/Ashkenazi chromosomes and to test the founder-effect hypothesis directly.

Table 3

Frequencies of *FES* Alleles in Ashkenazi and Non-Ashkenazi Persons Who Present or Do Not Present the BS Phenotype

Population	Allele[a]						n[b]
	C1	C2	C3	C4	C5	C6	
1. BS/Ashkenazi		.03	.88	.09			33
2. Non-BS/Ashkenazi	.04	.23	.39	.31		.03	100
3. BS/non-Ashkenazi	.02	.29	.40	.29			48
4. Non-BS/non-Ashkenazi	.06	.17	.42	.35	<.01	.01	214

[a] Blanks indicate that allele was absent from the sample. The frequencies of alleles in the non-BS/non-Ashkenazi chromosomes was determined by genotyping unrelated grandparents in 40 CEPH families. The putatively identical *FES* alleles in affected persons in consanguineous families were scored once only. Similarly, one BS/Ashkenazi chromosome from an individual whose parents are related (79MeDer) was counted only once. In multiplex families, data from a single affected from each family are included in this table.

[b] No. of chromosomes tested.

Consistent with the less tight linkage of *BLM* and *D15S116,* the association of the *D15S116* 7 allele with blm^{Ash} is weaker than the associations between the *FES* C3 and blm^{Ash} and between the *D15S127* 145-bp or 147-bp alleles and blm^{Ash}. The fact that only the *D15S116* 7 allele exhibits an increased association with blm^{Ash} is also consistent with the founder-effect hypothesis.

A small number of the *blm* alleles in the Ashkenazi Jewish population are probably derived from new mutations and hence would not be descended from the hypothetical founder. The carrier frequency of *blm* in the Ashkenazi population is ~1 in 110 (German et al. 1977). Assuming Hardy-Weinberg proportions, we calculate the gene frequency of *blm* in the Ashkenazim to be .00457. If the mu-

Table 4

Frequencies of *D15S127* Alleles in Ashkenazi and Non-Ashkenazi Persons Who Present or Do Not Present the BS Phenotype

	Allele[a]																							
Population	114	116	120/124	126	128	130	132	134	135	137	138	139	140	141	142	143	144	145	146	147	149	151	153	*n*
1. BS/Ashkenazi				.03	.03	.03			.03									.45	.03	.39				33
2. Non-BS/ Ashkenazi	.05			.04	.16	.07	.06	.02	.12	.09	.06	.05	.01	.06	.01	.03	.03	.10		.03	.01			100
3. BS/ Non-Ashkenazi	.07	.02			.20	.09	.19	.02		.04	.07	.02	.02		.02	.04		.13	.02		.02	.04		54
4. Non-BS/ non-Ashkenazi	.16	.01	.02	.01	.18	.06	.02	.03	.08	.11	.01	.05		.07		.06		.06		.03	.02	.01	.01	168

NOTE.—Notes to table 3 also apply to this table.

[a] Frequencies of alleles in the non-BS/non-Ashkenazi chromosomes were taken from Beckmann et al. (1993).

tation rate at *BLM* is 5×10^{-6} and if there have been 30 generations since the establishment of the Ashkenazi population, then 3.3% of the *blm* chromosomes should be from new mutations (see Subjects and Methods).

Linkage disequilibrium has been detected in a number of other genetically determined clinical disorders in which the disease-associated mutation is at an increased frequency in a specific population relative to most others: an association of a rare or infrequent haplotype with a disease-associated mutation has been identified in Friedreich ataxia in Cajuns (Sirugo et al. 1992), in diastrophic dysplasia and progressive myoclonus epilepsy in Finns (Hastbacka et al. 1992; Lehesjoki et al. 1993), in Wilson disease in Sardinians (Bowcock et al. 1994; Petrukhin et al. 1993; Thomas et al. 1994), in familial Mediterranean fever in Moroccan Jews (Aksentijevich et al. 1993), in familial dysautonomia (Blumenfeld et al. 1993), in idiopathic torsion dystonia (Ozelius et al. 1992), and in BS in Ashkenazi Jews (present study). In these examples, linkage disequilibrium can be interpreted as the consequence of founder effect because the populations in which the disease-associated mutation reached an increased frequency had originated during historic times and were genetically isolated.

Other examples of linkage disequilibrium have been documented in clinical disorders in which the causative mutation provides the heterozygous individual with an increased fitness over homozygous individuals under the selection of specific environmental agents. For example, certain mutations in the globin gene, which in the homozygous state can lead to serious hemoglobinopathies, can in the heterozygous state provide increased resistance to different forms of malaria (Flint et al. 1993). Given the low frequency of the *blm* mutation in the Ashkenazi Jewish population (and its much lower frequency elsewhere), we think it unlikely that it has ever anywhere conferred an advantage on heterozygotes. A similar argument can be made against a hypothesis in which selection acts at a locus tightly linked to *BLM*, an effect called *hitchhiking*.

The increased frequency of the *D15S127* 132-bp (as well as the 138-bp) allele in BS/non-Ashkenazi versus non-BS/non-Ashkenazi samples perhaps suggests that the mutant *blm* alleles on these chromosomes are identical. On the other hand, the BS/non-Ashkenazi chromosomes are from diverse populations, whereas the non-BS/non-Ashkenazi chromosomes are mainly from a sample of individuals of western European descent. Therefore, the frequency differences could be a sampling artifact.

Table 5

Frequencies of *D15S116* Alleles in Ashkenazi and Non-Ashkenazi Persons Who Present or Do Not Present the BS Phenotype

	Allele[a]														
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	*n*
1. BS/Ashkenazi				.06	.06	.03	.44	.13	.22		.06					32
2. Non-BS/Ashkenazi[b]	.05	.01	.01	.09	.07	.11	.26	.11	.12	.13	.02	.01		.02		94
3. BS/non-Ashkenazi[b]	.02	.06	.02	.07	.06	.07	.11	.13	.15	.13	.07	.04	.06		.02	54
4. Non-BS/non-Ashkenazi				.13	.21	.04	.19	.06	.06	.08	.09	.04	.02	.06	.02	52

NOTE.—Notes to table 3 also apply to this table.

[a] Frequencies of alleles in the non-BS/non-Ashkenazi chromosomes were taken from Beckmann et al. (1993).

[b] The sum of the allele frequencies is >1.00 because of rounding error.

Linkage disequilibrium has been employed both to refine the localization of disease-associated genes by estimating map distances (Hastbacka et al. 1992; Lehesjoki et al. 1993) and to isolate such genes by positional cloning (Kerem et al. 1989; The Huntington's Disease Collaborative Research Group 1993). Using linkage disequilibrium, we have estimated the map distance between *FES* and *BLM* to be 0.62 cM (see Subjects and Methods and the Appendix). This value is consistent with the map distance obtained from study of consanguineous families (German et al. 1994). As more flanking markers around *BLM* are identified, we will be able to test the usefulness of linkage disequilibrium in refining the localization of *BLM*.

As we have stated, linkage disequilibrium observed between *FES, D15S127, D15S116,* and *BLM* in Ashkenazi Jews with BS supports a founder-effect hypothesis. But, the linkage disequilibrium points to an interesting and fundamental effect of the population genetics of the Ashkenazim itself. From our genetic dissection of a small portion of chromosome 15 that flanks the *BLM* locus, all Ashkenazi Jews today who carry *blm* can be considered distant relatives, the equivalent of, say, 29th cousins. Finally, mapping of *BLM* by linkage disequilibrium is an extension of our earlier homozygosity mapping project that identified linkage between *FES* and *BLM,* an extension into an ethnic population completely different from the one that we studied originally, which was almost exclusively non-Ashkenazi Jewish. Linkage disequilibrium should help us localize *BLM* and hence bring us toward the ultimate goals of isolating *BLM* and defining the underlying biochemical defect in BS.

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Appendix

Following the arguments presented in Lehesjoki at al. 1993, let us assume that most mutation-bearing chromosomes in the population descended from a single ancestral chromosome along with allele A1 at a nearby locus. Then let α = the proportion of (current) mutation-bearing chromosomes descended from the assumed founder, π = the proportion of such chromosomes in which a recombinational event has *not* occurred, p_{norm} = the frequency of A1 in the general population, and p_{aff} = the frequency of A1 in the set of all mutation-bearing chromosomes. Then $p_{aff} = \alpha\pi+(1-\alpha\pi)p_{norm}$. Therefore $p_{aff} - p_{norm} = \alpha\pi(1-p_{norm})$ and $\alpha\pi = (p_{aff}-p_{norm})/(1-p_{norm})$.

Now, assume a mutation rate, μ, from normal to disease alleles, and let q equal the frequency of the disease allele in the population. Further, assume that the population has grown in a uniformly exponential manner, from a small size, for a total of t generations since the founder mutation occurred. Then mutation-bearing chromosomes with *new* mutations should constitute μt of all chromosomes or a proportion $\mu t/q$ of all mutation-bearing chromosomes.

Thus, α, the proportion of mutation-bearing chromosomes from the founder, will be $\alpha = 1-(\mu t/q)$. Now π can be estimated as a function of the recombination fraction between the disease and marker loci and the number of generations, t: $\pi = (1-\theta)^t$. Combining equations, we get $\alpha\pi = (1-\theta)^t(1-\mu t q^{-1})$ or $(1-\theta)^t = \alpha\pi/(1-\mu t q^{-1})$, which can be used to estimate θ.

We can apply these formulas to our BS/Ashkenazi and non-BS/Ashkenazi samples to obtain an estimate of the recombination fraction between *FES* and *BLM*. From table 3 we have $p_{aff} = .88$ and $p_{norm} = .39$. We have estimated q, the allele frequency of *blm,* to be .00457 (German et al. 1977). If the mutation rate at *BLM* is 5×10^{-6} and there have been 30 generations since the founder mutation occurred, then $(1-\theta)^{30} = .83054$. Solving this equation for θ, we get $\theta = .0062$.

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