

The Gene for Hereditary Bullous Dystrophy, X-Linked Macular Type, Maps to the Xq27.3-qter Region

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Summary

Bullous dystrophy, hereditary macular type (McKusick 302000), is an X-linked disorder and was originally described in a single kindred in the Netherlands by Mendes da Costa and Van der Valk in 1908. To determine the location of the bullous dystrophy gene, segregation studies were performed in this family and in a recently described Italian family. Using informative polymorphic markers, the gene could initially be localized on the Xq27-q28 region. No recombinants were noted with loci in Xq27.3-q28. Fine mapping places the bullous dystrophy locus distal to DXS102 (Xq26.3) in the Italian family and distal to DXS998 (Xq27.3) in the Dutch family.

Introduction

Hereditary bullous dystrophy macular type (HBD) (McKusick 302000 [1992]) is an X-linked recessive disorder characterized by the formation of bullae without evident trauma; hyper- and hypopigmentation; absence of hair at birth; and, in some cases, microcephaly, mild mental retardation, short conic fingers, and aberrations of the nails (van der Valk 1922; Carol and Kooij 1937; Hassing et al. 1980). Carrier females are found to be asymptomatic for any of the clinical features exhibited in patients. The disease was originally described by Mendes da Costa and van der Valk (1908). Recently, a second family with bullous dystrophy has been described by Lungarotti et al. (1994). Some clinical symptoms in HBD patients are similar to those observed in epidermolysis bullosa. Therefore, some authors classify HBD as an X-linked form of epidermolysis bullosa (Gedde-Dahl

and Anton-Lamprecht 1990). However, other authors do not share this view and argue that HBD should not be included as a type of epidermolysis bullosa, since blisters in HBD occur only spontaneously and cannot be provoked by trauma (Haber et al. 1986). The biochemical defect responsible for hereditary bullous dystrophy is unknown, and the location of the gene has not yet been determined.

Mapping of the HBD gene will be useful for carrier detection and may eventually lead to the isolation of the gene involved. In this paper we report linkage studies with probes covering the entire X chromosome, to obtain linkage data in the family described by Mendes da Costa and van der Valk (1908) and in the recently reported Italian family.

Subjects, Material, and Methods

Subjects

A genetic study encompassing the entire X chromosome was performed in the original bullous dystrophy family described by Mendes da Costa and van der Valk (1908). Patients showed typical atrichia at birth and from the age of 3 mo onward until the age of ~6 years, blisters developed on the skin. In the course of several years, hyperpigmentation intermingled with lenticular hypopigmentation developed, which was most prominent on the head and neck and extremities. Cases VI-1 and VI-6 showed mental retardation, and growth retardation was observed in patients VI-3 and VI-6.

The pedigree investigated in this study is shown in figure 1. Blood samples were obtained from 24 family members including four affected males and six obligate carrier females. A small aliquot of the blood sample was used for immortalization with Epstein-Barr virus, while the rest was used for DNA isolation. DNA of seven family members including one affected male and two obligate carriers of an Italian HBD family (Lungarotti et al. 1994) was also analyzed (fig. 2).

DNA Analysis

Genomic DNA was prepared from whole blood or cultured lymphoblasts by standard proteinase K diges-

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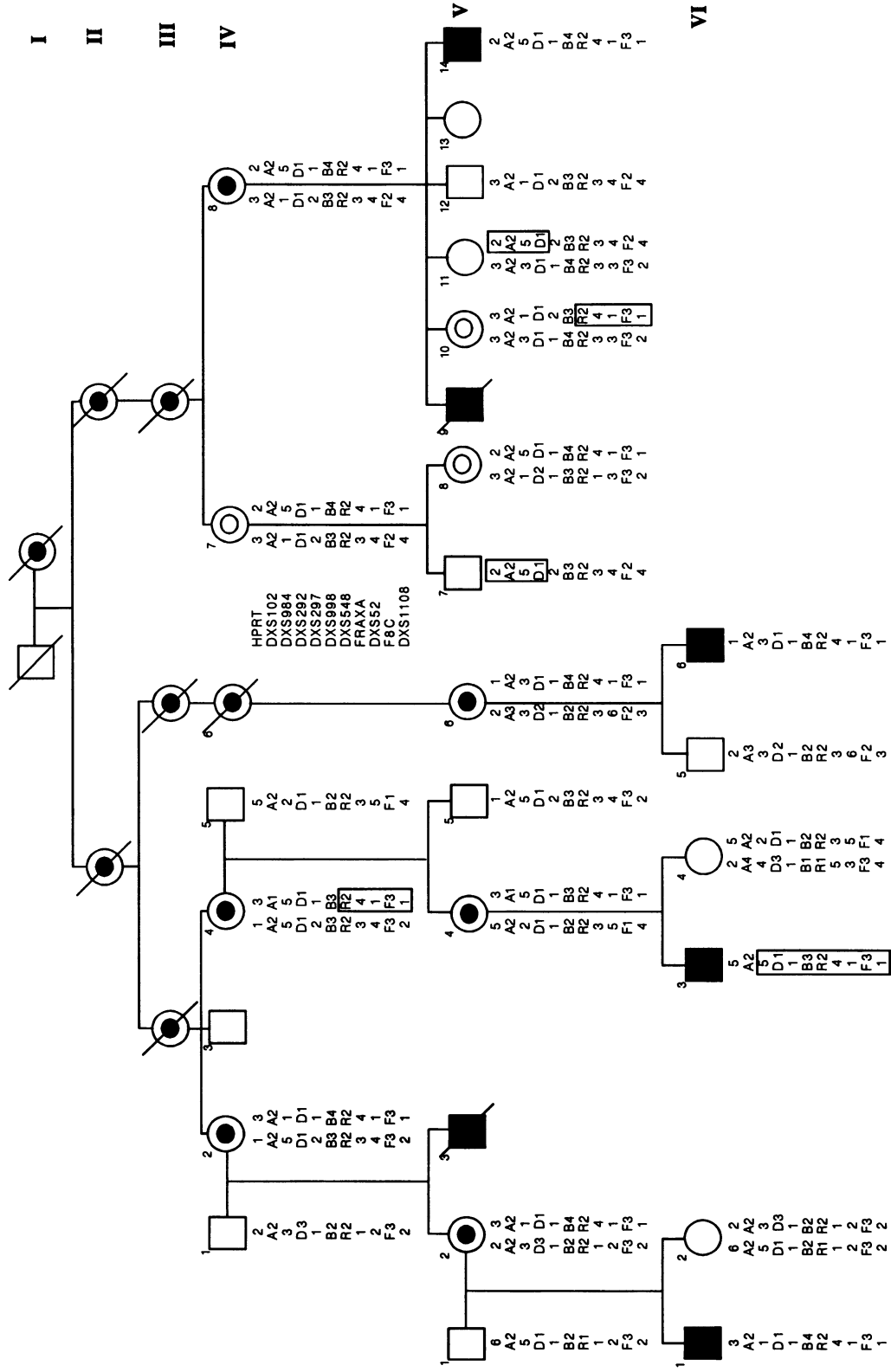


Figure 1 Dutch pedigree of the hereditary bullous dystrophy family used in this linkage study. Individuals' genotypes are shown for the loci HPRT, DXS102, DXS984, DXS292, DXS297, DXS998, FRAXA, DXS52, F8C, and DXS1108. Haplotype analysis is based on the minimum number of recombinants in the offspring. Boxes indicate part of the maternal risk chromosome inherited after a recombination event. Females with an unblackened inner circle are probable but not proven carriers on the basis of the observed haplotypes. In cases where the phase is unknown, alleles have been arranged to accommodate the fewest crossovers. Individual IV-3 was not cooperative.

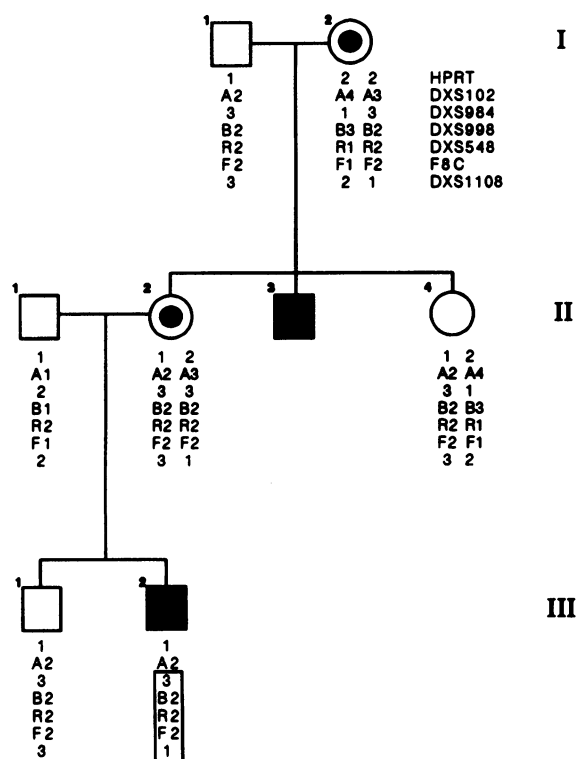


Figure 2 Fine mapping of the Xq26.1-ter region in the Italian HBD family. Patient III-2 inherited the region distal to DXS102 from his grandmother, confirming the location of the disease gene in the Xqter region. Boxes indicate part of the maternal risk chromosome inherited after a recombination event.

tion, phenol-chloroform extraction, and isopropanol precipitation. Thirty-three various DNA probes were used in this study to scan the entire X chromosome. Twenty-four of them were found to be informative and are summarized in table 1. For RFLP analysis, Southern blot analysis was performed using standard procedures (Sambrook et al. 1989).

The microsatellite markers were amplified according to standard procedures using ^{32}P - α -dATP, fluorescein-12-dUTP, or fluorescent primers. The PCR products were separated on polyacrylamide gels and visualized using autoradiography, an ALF DNA sequencer, and Fragment Manager version 1.1 of Pharmacia or the genescan mode of the Applied Biosystems automatic sequencer.

Amplification of the St14 minisatellite (Richards et al. 1991) was carried out in a total volume of 25 μl by using ~ 200 ng genomic DNA and 200 μM dNTPs. The products were separated on a 1.5% agarose gel.

Linkage Analysis

Two-point linkage analysis was performed using MLINK from the LINKAGE version 5.10 package

(Lathrop et al. 1984a, 1984b). A disease frequency of .00001 was assumed. Penetrance was considered to be complete, and no new mutations were allowed. Multilocus analysis was performed; however, no additional information was obtained, since no crossovers were detected distal to DXS998.

Results

Results of two-point lod scores for linkage between the HBD gene and X-chromosomal marker loci are summarized in table 1. Highly significant negative lod scores were obtained with loci on the short arm and the proximal long arm of the X chromosome. On the other hand, FRAXA and more distal marker loci showed close linkage with the HBD locus (table 1). We obtained a maximum lod score (Z_{max}) of 3.34 at a maximum recombination fraction (θ_{max}) of .00 for FRAXA (Xq27.3). DXS52, F8C, and DXS1108 (Xq28) also showed significant linkage with the disease (Z_{max} is 3.58, 2.07, and 3.93, respectively) at a θ of .00.

To locate the HBD gene in a more precise interval on the map we performed fine mapping using polymorphic markers distal to the hypoxanthine phosphoribosyl transferase locus (HPRT) in both the Dutch and the Italian HBD families. The informative markers in the Xq26-q28 region are shown in figures 1 and 2. Patient VI-3 inherited the maternal grandmother alleles except for the loci proximal to DXS984 (fig. 1).

Comparison of carriers IV-2 and IV-4 indicates that in their mother, carrier III-1, a recombination between DXS998 and the disease locus has occurred. This places the HBD locus distal to DXS998 in the Dutch pedigree.

In the Italian family a single recombination event localizes the HBD gene distal to DXS102 (fig. 2). This is in good agreement with the observed tight linkage with the Xq27.3-q28 marker loci.

Discussion

HBD is a rare X-linked disorder, originally described in only one family in the Netherlands. Recently a family with two patients with the characteristics of this syndrome has been identified in Italy (Lungarotti et al. 1994). The genealogical relationship between these patients and the described family is presently under investigation.

Our linkage data show that the HBD gene is located in the Xq27.3-28 region. The maximum two-point lod scores in this region are >2.0 which is evidence for linkage for an X-linked disease. Fine-mapping analysis of the region distal to HPRT on Xq26.1 in the Dutch family showed no recombinants between the disease locus and markers in the region distal to DXS998 in Xq27.3. The recombination shown in the Italian family in figure 2 places the HBD gene distal to DXS102. How-

Table I**Two-Point Linkage Data between the HBD Locus and Marker Loci**

Locus	θ						
	.00	.01	.05	.10	.20	.30	.40
DXS85	−∞	−4.51	−2.45	−1.60	−.81	−.40	−.15
DXS43	−∞	−3.28	−1.90	−1.31	−.75	−.42	−.19
KALLMAN	−∞	−4.08	−2.10	−1.32	−.65	−.33	−.13
DXS426	−∞	−4.72	−2.64	−1.77	−.94	−.49	−.20
DXS7	−∞	−1.11	−0.45	−0.20	−.01	.40	.04
DXS255	−∞	−5.91	−3.16	−2.03	−.98	−.45	−.15
DXS453	−∞	−2.98	−1.62	−1.06	−.54	−.28	−.11
DXS456	−∞	−2.21	−.88	−.38	.02	.14	.12
DXS454	−∞	−1.65	−.97	−.68	−.41	−.25	−.12
DXS458	−∞	−3.75	−1.75	−.97	−.32	−.06	.03
DXS3	−∞	−2.03	−.72	−.24	.12	.20	.15
DXS424	−∞	.24	.80	.93	.88	.67	.38
DXS425	−∞	−3.75	−1.74	−.96	−.30	−.05	.04
HPRT ^a	−∞	−4.61	−1.95	−.96	−.23	−.03	−.01
DXS102 ^a	−∞	−2.91	−1.01	−.35	.08	.17	.13
DXS984 ^a	−∞	−2.05	−.66	−.14	.22	.27	.18
DXS292	1.49	1.45	1.29	1.10	.74	.43	.19
DXS297	1.76	1.71	1.53	1.30	.87	.49	.18
DXS998 ^a	−∞	.98	1.43	1.41	1.09	.71	.34
DXS548 ^a50	.48	.41	.32	.18	.08	.03
FRAXA	3.34	3.26	2.95	2.55	1.76	1.01	.37
DXS52	3.58	3.50	3.18	2.77	1.96	1.17	.49
F8 ^a	2.07	2.02	1.81	1.54	1.04	.59	.24
DXS1108 ^a	3.93	3.85	3.51	3.08	2.22	1.38	.63

^a Also used in the Italian family; lod scores are combined.

ever, since carrier II-2 is not informative for DXS984, DXS998, DXS548, or F8C, a more distal recombination cannot be excluded.

We tested all available markers distal to HPRT with a known map position (Schlessinger et al. 1993). Markers informative in the Dutch and Italian family were used in the fine-mapping analysis, and, consequently, a more precise location for HBD cannot be obtained unless new HBD families are identified.

The chromosomal region Xq27.3-q28 is known to contain many disease genes. Superficially there are analogies in symptoms presented by HBD and incontinentia pigmenti (IP-2) patients. The IP-2 locus has been mapped to Xq28, but the disease is X-linked dominant in females and lethal in males, which makes it an unlikely candidate locus (Sefiani et al. 1989, 1991; Smahi et al. 1994).

A plausible candidate gene for HBD in this chromosomal region is biglycan I, located between DXS52 and F8C (Schlessinger et al. 1993). The gene encodes a small proteoglycan found in many connective tissues and is also expressed in epithelial cells. The dermatological symptoms in HBD patients could be explained by dysfunction of this proteoglycan. Mutation analysis of the gene is possible, now that the sequence of the relevant

regions of the gene is known (Fisher et al. 1991). No mutations in this gene have been detected for any of the other diseases involving connective tissue abnormalities assigned to Xq28: chondrodysplasia punctata, dyskeratosis congenita, and incontinentia pigmenti (Das et al. 1994). The localization of the HBD locus to a limited region on the X chromosome opens the way for carrier detection and identification of the gene affected in this disease.

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