Fine mapping of the dyskeratosis congenita locus in Xq28

S W Knight, T Vulliamy, G L Forni, D Oscier, P J Mason, I Dokal

Abstract

Dyskeratosis congenita (DC) is characterised by reticulate skin pigmentation, mucosal leucoplakia, and nail dystrophy. Bone marrow failure occurs in 50% of patients and is the principal cause of early mortality. In the majority of families the pattern of inheritance of DC is compatible with an X linked recessive trait. The locus for the X linked recessive form of DC has been linked to Xq28. We have now extended our earlier studies by investigating five families with additional Xq28 polymorphic markers; analysis of recombination events in these families has located the DC1 locus between GABRA3 and DXS1108, an interval of approximately 4 Mb.

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Key words: dyskeratosis congenita; X linked; Xq28.

Dyskeratosis congenita (DC) (MIM 305000) is an inherited multisystem disorder which is characterised by the early manifestation of reticulate skin pigmentation, mucosal leucoplakia, and nail dystrophy, usually below the age of 15 years.¹ A variety of non-cutaneous features have been reported, including epiphora, early dental loss, and skeletal and gastrointestinal abnormalities. Bone marrow failure occurs in approximately 50% of cases and there is also a predisposition to malignancy, especially epithelial carcinomas of the gastrointestinal tract and skin.¹ The finding of chromosomal rearrangements, particularly in fibroblasts, suggests that DC is a chromosomal instability disorder,² although the evidence for an increased susceptibility to clastogenic agents is disputed.³ In the majority of reported families the inheritance of DC is compatible with an X linked recessive disorder.¹ Familial and sporadic female cases of DC have been reported with possible autosomal dominant (MIM 127550) and recessive (MIM 22430) modes of inheritance, a milder clinical phenotype having been observed in the autosomal dominant cases.¹ We propose to call the major X linked DC locus DC1. Analysis of polymorphic markers within the long arm of the X chromosome in one large pedigree assigned the X linked DC1 locus to Xq28⁴ and this linkage was subsequently confirmed in three other families.⁵ Here we present a refinement of the linkage analysis using additional polymorphic markers within Xq28, which allows the region of the DC1 locus to be delimited.

Subjects and methods

FAMILIES

A total of five families from the Dyskeratosis Congenita Registry were used in the linkage analysis; for each the inheritance of DC was consistent with an X linked recessive disorder (fig 1). Family 001 has been described previously and linkage to DXS52 reported,^{5,6} although this pedigree is now updated with new members and subject IV.3 confirmed as being affected. Family 007 is related to the pedigree for which linkage analysis was originally performed.⁴ At risk subjects were considered to be unaffected if they were over 15 years of age and without nail dystrophy, leucoplakia, or skin abnormalities or any other recognised symptoms of the disease. Male subjects below the age of 15 were classified as unknown unless definite clinical signs were ascertained by the authors. In family 002, the obligate carrier I.2 had the odd dystrophic nail on the hands and feet together with slight abnormal pigmentation around one ankle. Those people who were available for use in the linkage analysis are indicated with an asterisk in fig 1.

METHODS

DNA was extracted from leucocyte pellets in the standard way.⁷ The primers and PCR conditions used for the following microsatellite markers were taken from the respective references DXS1113,⁸ DXS1684,⁹ GABRA3,¹⁰ and DXS1108.¹¹ PCR products were analysed on 6% urea polyacrylamide gels after ³²P dCTP had been added to the reactions. The VNTR DXS52¹² was amplified and the products were analysed on an agarose gel. The diallelic markers G6PD¹³ and F8C¹⁴ were investigated by amplification followed by digestion with the appropriate restriction enzymes and analysis on an agarose gel. The allele frequencies for the polymorphic markers were taken from the GDB 6.0. The DC1 locus was tested assuming that the disease was an X linked recessive trait with an allele frequency of the affected allele of 0.001 with complete penetrance. The family linkage data were managed using the IGD/XPED database system.¹⁵ Linkage analysis was performed using the MLINK programme of the LINKAGE package.¹⁶

Results

The pairwise linkage analysis between DC1 and each polymorphic marker resulted in a maximum lod score with DXS52 ($Z_{max} = 4.4$, $\theta = 0$) (table 1). No linkage disequilibrium

Department of
Haematology, Royal
Postgraduate Medical
School and
Hammersmith
Hospital, Du Cane
Road, London
W12 0NN, UK
S W Knight
T Vulliamy
P J Mason
I Dokal

Ente Ospedaliero
Ospedali Galliera,
Mura della
Capuccine 14, 16128
Genova, Italy
G L Forni

Department of
Haematology, Royal
Bournemouth
Hospital,
Bournemouth, UK
D Oscier

Correspondence to:
Dr Knight

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Figure 1 Pedigrees of families with dyskeratosis congenita.

Table 1 Pairwise lod scores between DC1 and six polymorphic marker loci

Locus	Recombination fraction θ						
	0.00	0.010	0.050	0.100	0.200	0.300	0.400
DXS1113	— ∞	−0.498	0.691	1.023	1.053	0.795	0.418
DXS1684	— ∞	−2.730	−0.803	−0.119	0.328	0.375	0.244
DXS52	4.405	4.327	4.003	3.577	2.663	1.690	0.744
G6PD	3.097	3.041	2.809	2.509	1.871	1.192	0.524
F8C	1.896	1.864	1.734	1.565	1.200	0.800	0.381
DXS1108	— ∞	1.755	2.215	2.204	1.835	1.281	0.637

between DC1 and any DXS52 allele was observed. In family 006, three recombination events were identified when the phase of the markers was inferred and the genotypes of the grandparents deduced (fig 2). Recombination events were inferred between DXS52 and DXS1684 in II.2 and between F8C and DXS1108 in II.4. Another recombination was observed when the GABRA3 marker was analysed in II.2 between DXS52 and GABRA3, which enabled the centromeric boundary of the DC1 locus to be defined (fig 2). The affected subject III.5 inherited the recombinant chromosome from his mother II.4, thus allowing for the telomeric boundary of the DC1 locus to be determined as DXS1108 (fig 2). On the basis of the genetic linkage analysis in this family we would predict that the daughter III.3 is a carrier of the disease. In family 002, in which there is cosegregation of the disease and DXS52, one further recombinant chromosome was observed between DXS1684 and DXS52 in II.8. In this case, the GABRA3 marker was uninformative and no further refinement of the location of the DC1 locus was possible. In

summary the most probable location for the DC1 locus is between GABRA3 and DXS1108.

Discussion

We report the fine genetic mapping of the X linked form of dyskeratosis congenita within Xq28. The DC1 locus is now delimited to between the polymorphic markers GABRA3 and DXS1108, a region of 4 Mb according to the recent X chromosome map.¹⁷ In a report of a family showing X linked inheritance of DC, there is evidence of recombination between G6PD deficiency and DC.¹⁸ This raises the possibility that the G6PD gene represents a boundary to the DC1 locus. The absence of recombinations between the marker DXS52 and dyskeratosis congenita in any reported pedigree^{4,5} emphasises the usefulness of this marker in the identification of carriers and early diagnosis of people considered to be at risk.⁶

A number of candidate genes from within Xq28 have been investigated as the possible DC1 gene. Single strand conformational polymorphism analysis of the biglycan and p55 genes detected no mutations.^{19,20} Sequencing of the QM gene in DC patients showed no mutations²¹ and no gross differences were detected in the DNase I-like gene DNL1L from DC patients.^{22,23} The melanoma associated antigen genes (MAGE), which occur in three distinct clusters within Xq27-Xq28, have been suggested as possible candidates for the DC1 locus.²⁴ This report enables the exclusion of some of the MAGE genes as candidates for the DC1 gene. However, the MAGE-2, MAGE-

Figure 2 Pedigree of family 006 showing the haplotypes of which the phase has been inferred minimising the number of recombinations. The bracketed haplotypes of the grandparents were deduced from their offspring. The affected haplotype has been boxed.

3, MAGE-6, and MAGE-12 genes remain positional candidates.²⁴

In conclusion, this work has enabled us to refine the location of the DC1 locus which will greatly aid the subsequent positional cloning of the DC1 gene.

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