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ORIGINAL INVESTIGATION

Hans Eiberg · Jan Mohr

Dombrock blood group *(DO)*: assignment to chromosome 12p

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Abstract The Dombrock blood group system (*DO*) is a common polymorphism in Caucasians, represented by two red cell antigen alleles. In a linkage study in our family material of 832 families from the Copenhagen area, we found a strong indication of tight linkage with the two flanking DNA polymorphisms D12S358 (z = 7.66; at θ_M = 0.001, θ_F = 0.031) and D12S364 (z = 8.53; at θ_M = 0.068, θ_F = 0.031). *DO* is assigned to the region 12p13.2–12p12.1 by physically localised markers.

Introduction

The Dombrock antibody DO(a) was detected in a transfused patient, Mrs. Dombrock (Swanson et al. 1965). Later, the antibody DO(b) was also described (Molthan et al. 1973). The antigens DO^a and DO^b appear to be well developed at birth: they have been found in normal strength in cord blood (Swanson et al. 1965; Molthan et al. 1973). The genetics of the Dombrock blood group system (MIM 110600) have been described by Tippett (1967) and Tippett et al.(1972). The gene was not sited at any blood group locus known at the time (Swanson et al. 1965; Tippett et al. 1972; Molthan et al. 1973). The gene frequencies were calculated on the assumption that the antigen DO^a is a dominant character (the allele DO*A). The gene frequency of DO*A in Caucasians, p = 0.420, and in Negroes, p = 0.257, make the system a useful marker in linkage studies (Swanson et al. 1965; Tippett et al. 1972). However, antibodies to the two types of antigens are very rare, and only a few linkage studies of the blood group Dombrock have been made. Two groups have found positive lod scores to the markers *MNS* and *GC*. Tippett et al. (1972) found a loose linkage between *DO* and *MNS* (z = 1.83 at $\theta_{M=F}$ = 0.35). Olaisen et al. (1979) have presented data indicating possible linkage of *DO* to *GC*. Later the *DO:MNS* and *DO:GC* linkage could not be supported (Lewis et al. 1978, 1983; Mohr et al. 1989). The Dombrock system has been tentatively assigned to chromosome 1 by an apparent linkage with *PGD*, maximum lod score z = 3.56 at $\theta_{M=F}$ = 0.23 (Lewis et al. 1978, 1983). Data from Mohr and Eiberg (1985) could not confirm this linkage (z = –0.88 at $\theta_{M=F}$ = 0.20) and excluded linkage of *DO* to 29 other polymorphic systems.

In the present paper we indicate a linkage of *DO* to chromosome 12p (maximum lod score of z = 7.66; $\theta_{M\neq F}$ to the marker D12S358 and z = 8.53 $\theta_{M\neq F}$ to the marker D12S364, Table 1). These two markers flank the *DO* locus. *DO* is excluded from other chromosomes by exclusion mapping with 448 polymorphic systems.

Materials and methods

Family data

The family material consists of 832 families with at least four children living in the Copenhagen area. Serum, erythrocytes and lymphocytes were isolated from all blood samples and frozen for later typing (Eiberg et al. 1989). DNA was extracted from Epstein-Barr virus-transformed frozen lymphocytes. DNA amplification and restriction fragment length polymorphism (RFLP) analysis was done by standard methods (Eiberg et al. 1989). The typing for *DO* was done as described by Tippett (1967) in capillary tubes. The red blood cells were pretreated with Coombs before the direct antigen-antibody test. Since the anti-DO^a was in limited supply, only children with at least one DO^a-positive parent were typed in most cases.

Linkage analysis

A total of 80 classical markers (blood groups *ABO*, *MNS*, *JK*, *P* etc., serum and protein polymorphisms *HP*, *PLG*, *ACP1*, *GLO* etc., and physiological markers *PTC*, *BEY2*, *HCL2* etc.) were used for our primary detection of linkage (Eiberg et al. 1989), as well as 66 RFLP and 302 polymerase chain reaction systems. Two-point lod scores for an initial exclusion mapping were obtained with the computer program LIPED (Ott 1973). The lod scores $z(\theta_M = \theta_F)$, maximum

H. Eiberg (✉) · J. Mohr
Institute of Medical Biochemistry and Genetics,
Department of Genetics, Building 24.4, Blegdamsvej 3,
DK-2200 Copenhagen N, Denmark
Tel.: +45-35-32-78-29; Fax: +45-31-39-33-73;
e-mail he@rclink.imbg.ku.dk

Table 1 Lod scores between *DO* and chromosome 12 loci in 22 informative families

Marker	Informative families	Recombination fraction ($\theta_{M=F}$)						
		0.01	0.05	0.19	0.20	0.30	z_{max} at	θ(M; F)
D12S99	4	–3.79	–0.65	0.39	0.92	0.74	1.42	0.093; 0.500
D12S89	20	2.47	6.79	7.50	6.24	3.82	7.88	0.044; 0.143
D12S1697	6	2.79	4.32	4.48	3.76	2.46	5.17	0.001; 0.139
D12S358	7	7.40	7.27	6.52	4.69	2.78	7.66	0.001; 0.031
D12S364	7	7.95	7.89	7.22	5.47	3.42	8.53	0.068; 0.001
D12S62	21	12.62	13.85	13.01	9.91	6.08	13.91	0.054; 0.030
D12S87	8	–10.84	–3.76	–1.22	0.44	0.62	1.28	0.160; 0.500
Chromosome 1								
D1S243	6	–11.76	–5.71	–3.32	–1.30	–0.45	0.00	0.500; 0.500
D1S214	7	–16.46	–7.76	–4.36	–1.54	–0.45	0.00	0.500; 0.500
PGD	13	–18.57	–8.61	–4.76	–1.62	–0.43	0.00	0.500; 0.500
Chromosome 4								
GC	87	–93.11	–45.37	–26.53	–10.64	–3.99	0.00	0.500; 0.500
MNS	179	–191.04	–88.50	–48.81	–16.61	–4.57	0.00	0.500; 0.500

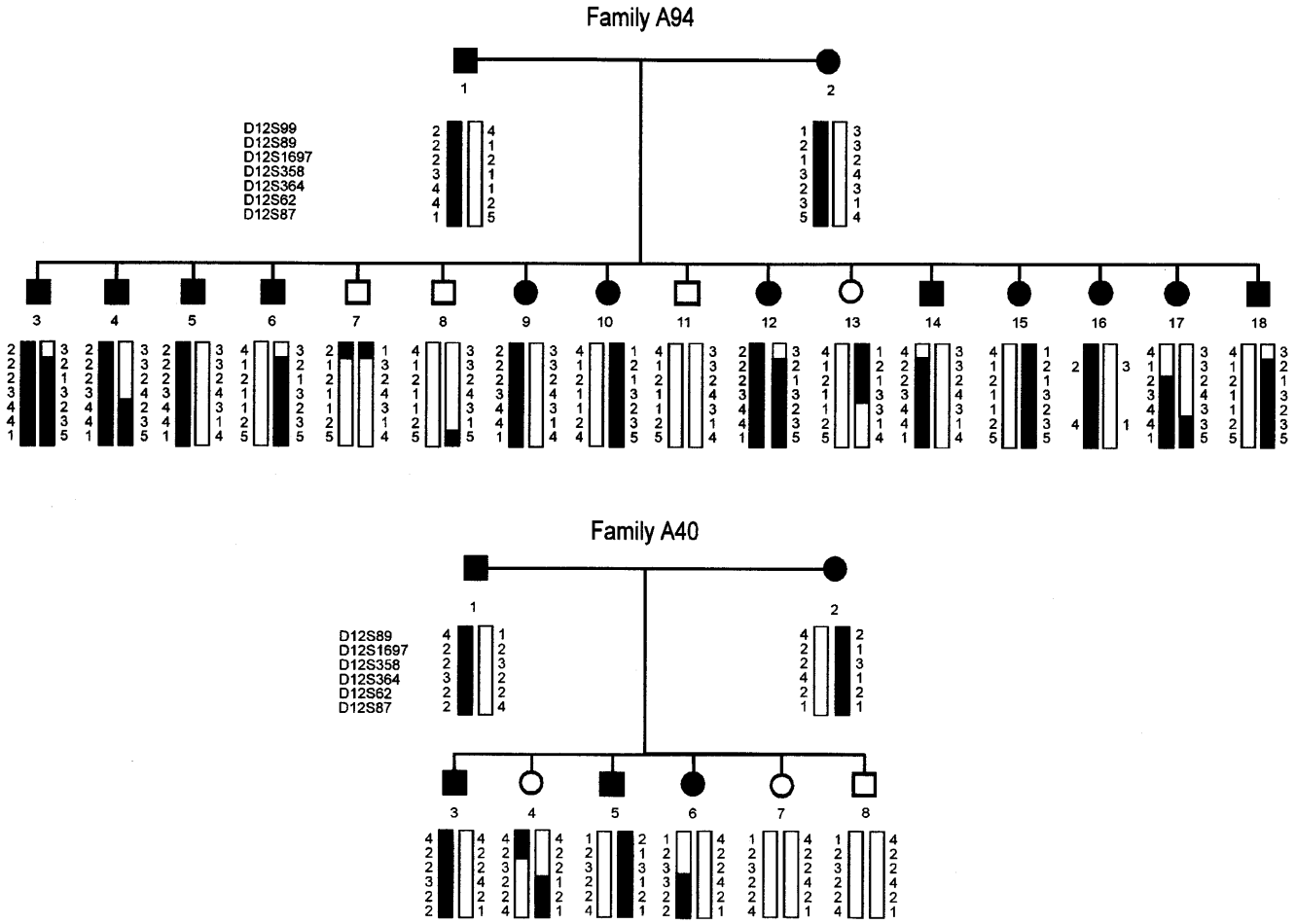

Fig. 1 Two families segregating for *DO*. From a total of seven families we found the order of chromosome 12 markers to be: D12S99–D12S89–D12S1697–D12S358–*DO*–D12S364–D12S62–D12S87. Do(a+) persons are indicated by *black symbols*

lod scores $z_{max}(\theta_M \neq \theta_F)$ and multipoint lod scores was calculated by the program FASTLINK (Schäffer et al. 1994). Exclusion mapping was done with the program EXCLUDE (Edwards 1987). DO*a was considered as a dominant allele with complete penetrance and a gene frequency of 0.40. The DNA markers D12S99, D12S87, D12S89, D12S62 (Weissenbach et al. 1992; Gyapay et al. 1994), D12S1697, D12S358 and D12S364 (Dip et al. 1996) were used for linkage studies to narrow down the area for *DO*.

Results

Our first effort to map *DO* was by exclusion mapping with 80 markers in 120 informative families and with 368 markers in 5 informative families (part of the 120 families) all from the Copenhagen family material (families 604–1505). This material was collected in 1973 for linkage studies of common polymorphisms. By this approach only chromosomal region 12p stood out as a possibility (100% possibility with or without D12S89). Further, the computer program LIPED gave a lod score of $z = 3.41$ ($\theta_{M=F} = 0.1$) to D12S89. We then typed 17 large families for the DNA microsatellite markers D12S89, D12S62, and D12S99 to consolidate the linkage. Data from eight persons in 7 families favour the following order: D12S89-*DO*-D12S62. These 7 families were used to narrow down further the presumed location of the *DO* locus, with new polymorphic marker systems from the Généthon database (D12S1697, D12S358 and D12S364). Two families with recombinations between D12S1697 and D12S358 are shown in Fig. 1. The multipoint lod score was calculated with markers positioned in accordance with the Généthon map. The markers and distances used for the multipoint analysis were: D12S99–(0.10)–D12S89–(0.025)–D12S1697–(0.025)–D12S358–(0.025)–D12S364–(0.025)–D12S62–(0.1)-D12S87. The final localisation of *DO* when all families were included, was calculated to be D12S99–D12S89–D12S1697–*DO*–D12S358–D12S364–D12S62–D12S87 using the LINKMAP program of the FASTLINK package, with a multipoint lod score of $z = 19.43$ (Fig. 2).

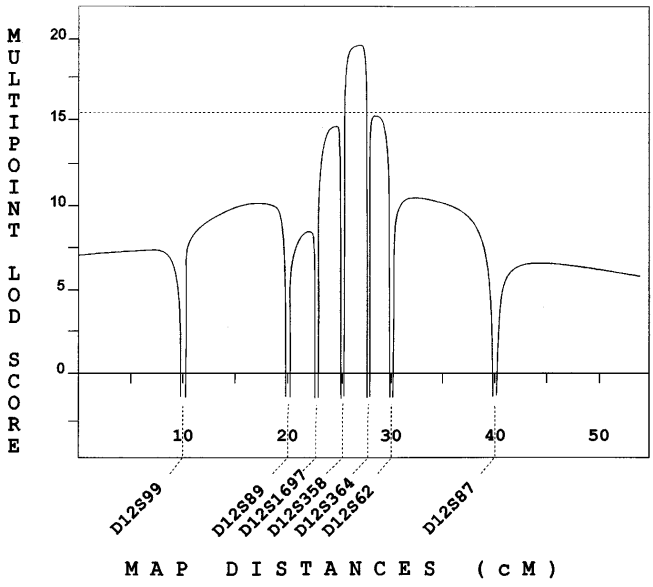

Fig. 2 Multipoint analysis with seven markers from chromosome 12q using 22 families informative for *DO*. The *horizontal dashed line* indicates the significance level for exclusion (odds 1:1000). *DO* is mapped between the markers D12S358 and D12S364

Discussion

By linkage analysis we have assigned the gene *DO* to chromosome 12p, with a lod score of $z = 13.91$ ($\theta_{M \neq F}$) for the DNA microsatellite marker D12S62. The most likely order was D12S89–D12S1697–D12S358–*DO*–D12S364–D12S62–D12S87 (Fig. 2). This region (Holt et al. 1992; Raeymaekers et al. 1995) is flanked by the regionally assigned markers *PRB3* (12q13.2; Mamula et al. 1985) and *KRAS2* (12q12.1; Sakaguchi et al. 1983), which maps *DO* to 12p13.2–12q12.1. The proposed linkage of *DO* to *PGD* on chromosome 1 ($z = 3.56$ at $\theta_{M=F} = 0.23$)(Olaisen et al. 1979) was not later supported by Mohr and Eiberg (1985) and in our present study not supported by the markers D1S243, D1S214 and *PGD*. Furthermore the markers *MNS* and *GC* could not support linkage of *DO* to chromosome 4.

The surface antigen loci *CD9*, *CD27* and *CD4* have been assigned to chromosomal region 12pter–12p11.2, but none of these are obvious candidates for *DO*. Linkage analysis and physical mapping (Raeymaekers et al. 1995) have localised these genes outside our localisation of the *DO* locus.

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